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**(54) Title: ORGANIC ANION TRANSPORTER GENES AND PROTEINS**

[illegible]

**(57) Abstract:** This invention relates to novel human organic anion transporter polypeptides and genes encoding such polypeptides. The invention is directed towards the isolation, characterization and use of human organic anion transporter polypeptides for pharmacological screening of substrates, analogues of substrates, and modulators of human organic anion proteins. This invention also provides cell cultures, which stably contain nucleic acid sequences encoding human organic anion transport polypeptides. Such cell lines are useful for screening compounds *in vitro* to identify and characterize modulators, inhibitors and substrates of human organic anion transporter polypeptides.

**ORGANIC ANION TRANSPORTER GENES AND PROTEINS****Related Applications**

This application claims the benefit of U.S. Serial No. 60/143,771, entitled  
5 "Organic Anion Transporter Genes and Proteins," which was filed July 12, 1999 and  
which is incorporated by reference herein in its entirety, including figures.

**Field of the Invention**

The present invention relates to specific proteins as well as recombinant versions  
10 of these proteins which are organic anion transporters. These proteins include human  
membrane proteins preferentially found in kidney and liver cells. The present invention  
also relates to nucleotide sequences encoding these novel organic anion transporters. In  
another aspect, the present invention relates to methods for using these proteins as *in vitro*  
screening agents to identify substrates for the proteins and inhibitors which block transport  
15 activity. In yet another aspect, these proteins may be used in *in vitro* assays to predict  
drug pharmacokinetics, drug distribution and drug toxicity. The invention also discloses  
antibodies that specifically bind to these proteins and which may be used in diagnostic  
assays.

**20 Background of the Invention**

The following describes certain relevant art, none of which is admitted to be prior  
art to the inventions described herein.

The liver and kidney are two organs that can extract a variety of organic anions  
from circulation, including endogenous compounds such as bile acids and bilirubin and  
25 xenobiotics such as sulfobromophthalein (BSP) and *p*-aminohippurate (PAH). The liver  
and kidney thus have the critical functions of bile secretion, detoxification, and drug  
elimination. Since the cell membrane represents a hydrophobic barrier that prevents influx  
of charged or hydrophilic molecules, hepatocytes and kidney epithelial cells express  
proteins on their basolateral membranes to facilitate transport of organic anions into the  
30 cell. The liver and kidney contain membrane proteins that transport very specific  
compounds such as taurocholic acid or prostaglandins. However, they also have proteins  
that exhibit a wider substrate specificity. Recently, transporters of the latter type have  
been cloned from rat liver and kidney. One such protein is called the organic anion

transporting polypeptide, oatp (Jacquemin et al. (1994) *PNAS* 91:133-37). This protein belongs to a family of related transporters, members of which consists of oatp1, oatp2, oatp3, and prostaglandin transporters. These proteins share homology at the amino acid level (Noe et al. (1997) *PNAS* 94: 10346-50; Kanai et al. (1995) *Science* 268: 866-69; Abe  
5 et al. (1998) *J. Biol. Chem.* 273: 22395-401). When expressed in *Xenopus* oocytes or in mammalian cells, members of the oatp family were shown to transport many types of organic anions including taurocholic acid, BSP, and conjugated steroid hormones. The related prostaglandin transporters show high affinity transport of prostaglandins.

Shortly after the cloning of oatp1, members of another organic anion transporter  
10 family, OAT1 and OAT2, were isolated from rat kidney and liver, respectively (Sekine et al. (1997) *J. Biol. Chem* 272: 18526-29; Sekine et al. (1998) *FEBS Letters* 429: 179-82). Rat OAT1 and OAT2 are homologous to each other but show no significant homology to members of the oatp family. However, they show some homology to the family of organic  
15 cation transporters (OCTs), suggesting that these two families of proteins share a common origin. Rat OAT1 and OAT2 can transport many different organic anions. OAT1, when expressed in oocytes, can transport *p*-aminohippurate (PAH), methotrexate, and glutarate. OAT1 is most likely the molecular entity responsible for the classical *p*-aminohippurate/ $\alpha$ -ketoglutarate exchanger found in the kidney proximal tubule. OAT2, expressed  
predominantly in the liver, has been shown to transport organic anions such as salicylate,  
20 methotrexate, and  $\alpha$ -ketoglutarate. Therefore, OAT1 and OAT2 belong to yet another family of multi-specific organic anion transporters distinct from the oatp family.

### Summary of the Invention

The present invention is directed to a group of polypeptides, preferentially  
25 expressed in the liver and kidneys of humans and which have activity as organic anion transporters. We refer to these polypeptides as "human organic anion transporters" or "hOAT polypeptides". These proteins and their properties are described in detail below.

The invention also concerns, nucleic acids encoding hOAT polypeptides, cells,  
tissues and animals containing such nucleic acids, antibodies to the polypeptides, assays  
30 utilizing the polypeptides, and methods relating to all of the foregoing.

A first aspect of the invention features an isolated, enriched, or purified nucleic acid molecule encoding an hOAT polypeptide or encoding a fragment of an hOAT polypeptide.

In preferred embodiments the isolated nucleic acid comprises a nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof, a nucleic acid sequence that hybridizes to the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof.

The nucleic acid may be isolated from a natural source by cDNA cloning or subtractive hybridization; the natural source may be mammalian (human) blood, semen, or tissue and the nucleic acid may be synthesized by the triester or other method or by using an automated DNA synthesizer. Preferably, the nucleic acid is isolated from mammalian kidney or brain, most preferably from liver.

Individual clones isolated from a cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones can be obtained directly from total DNA or from total RNA. The cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately  $10^6$ -fold purification of the native message. Thus, purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

In another preferred embodiment, the nucleic acid molecules of the invention comprise nucleotide sequences that (a) have the nucleic acid sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof, or encode polypeptides having the full-length amino acid sequences set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof; (b) is the complement of the nucleotide sequence of (a); (c) hybridizes under highly stringent conditions to the nucleotide molecules of (a) and encodes a naturally occurring hOAT polypeptide; (d) encodes an hOAT polypeptide having the full-length amino acid sequence of the sequence set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12 except that it lacks one or more of the domains selected from the group



consisting of an extracellular domain, a transmembrane domain, and an intracellular domain; or (e) is the complement of the nucleotide sequence of (d).

The nucleic acid molecules of the invention are isolated, enriched, or purified preferably from a mammal, more preferably from a human.

5 Hybridization techniques may be used to isolate a nucleic acid molecule of interest. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Stringency is controlled by varying salt or denaturant concentrations. Highly stringent conditions may mean conditions that are at least as stringent as the following: hybridization in 50% formamide, 5x SSC, 50 mM NaH<sub>3</sub>PO<sub>4</sub>,  
10 pH 6.8, 0.5% SDS, 0.1 mg/mL sonicated salmon sperm DNA, and 5x Denhart solution at 42 °C overnight; washing with 2x SSC, 0.1% SDS at 45 °C; and washing with 0.2x SSC, 0.1% SDS at 45 °C. Those skilled in the art will recognize how such conditions can be varied to vary specificity and selectivity.

Particularly preferred embodiments of this aspect of the invention are naturally or  
15 non-naturally occurring variants of the nucleic acids of the invention. Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or  
20 non-conservative amino acid substitutions, deletions or additions. Such nucleic acid molecules are identifiable as being able to hybridize to or which are at least about 60-65% percent identical, preferably at least about 70-75% percent identical, more preferably at least about 80-83% percent identical, and even more preferably at least about 87-95% percent identical to the nucleic acid molecules shown in SEQ ID NO:1, SEQ ID NO:2,  
25 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

Among the particularly preferred embodiments of the invention in this regard are nucleic acid molecules encoding polypeptides having the amino acid sequences set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12; variants, analogs, derivatives and fragments thereof, and fragments of the variants,  
30 analogs and derivatives.

Further particularly preferred in this regard are nucleic acid molecules encoding OAT variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence for the hOAT polypeptides of SEQ ID

NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the OAT polypeptides. Also especially preferred in this regard are conservative substitutions. In one embodiment, the polypeptide encoded by the nucleic acid molecule is at least about 30-35%, preferably at least about 40-45%, more preferably at least about 50-55%, even more preferably at least about 60-65%, yet more preferably at least about 70-75%, still more preferably at least about 80-85%, and most preferably at least about 90-95% or more identical to the amino acid sequence of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

In still another embodiment, the nucleic acid molecule encodes a naturally occurring variant of the polypeptide of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12 and hybridizes under stringent conditions to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, respectively.

Further preferred embodiments are variants of the herein described nucleic acid molecules in which the transporting activity of the polypeptides is altered. Such alterations can result in either increased, or decreased transport of substrates recognized by hOAT polypeptides. Further, variants can be made that result in OAT polypeptides which are no longer responsive to cellular signals, for example, protein kinase C deactivation of transport.

Especially preferred are nucleic acid molecules encoding OATs which are expressed on the basolateral (sinusoid) membrane of hepatocyte cells.

Included in the invention are all possible nucleic acid sequences that encode the amino acid sequences of the hOAT polypeptides of the invention. As it is recognized that alternate codons will encode the same amino acid for most amino acids due to the degeneracy of the genetic code, the sequences of this aspect includes nucleic acid sequences utilizing such alternate codon usage for one or more codons of a coding sequence. For example, all four nucleic acid sequences GCT, GCC, GCA, and GCG encode the amino acid, alanine. Therefore, if for an amino acid there exists an average of three codons, a polypeptide of 100 amino acids in length will, on average, be encoded by  $3^{100}$ , or  $5 \times 10^{47}$ , nucleic acid sequences. Thus, a nucleic acid sequence can be modified

(e.g., a nucleic acid sequence from an hOAT as specified above) to form a second nucleic acid sequence encoding the same polypeptide as encoded by the first nucleic acid sequence using routine procedures and without undue experimentation. Thus, all possible nucleic acid sequences that encode the amino acid sequences of the hOAT polypeptides of the invention are included in the present invention and are described, as if all were written out in full, taking into account the codon usage.

The alternate codon descriptions are available in common textbooks, for example, Stryer, BIOCHEMISTRY 3<sup>rd</sup> ed., and Lehninger, BIOCHEMISTRY 3<sup>rd</sup> ed. Codon preference tables for various types of organisms are available in the literature. Because of the number of sequence variations involving alternate codon usage, for the sake of brevity, individual sequences are not separately listed herein. Instead the alternate sequences are described by reference to the natural sequence with replacement of one or more (up to all) of the degenerate codons with alternate codons from the alternate codon table, preferably with selection according to preferred codon usage for the normal host organism or a host organism in which a sequence is intended to be expressed. Those skilled in the art also understand how to alter the alternate codons to be used for expression in organisms where certain codons code differently than shown in the "universal" codon table.

In a second aspect, the invention relates to a nucleic acid vector comprising a nucleic acid molecule encoding an hOAT polypeptide and a promoter element effective to initiate transcription in a host cell.

Those skilled in the art would recognize that a nucleic acid vector can contain many other nucleic acid elements besides the promoter element and the hOAT nucleic acid molecule. These other nucleic acid elements include, but are not limited to, origins of replication, ribosomal binding sites, nucleic acid sequences encoding drug resistance enzymes or amino acid metabolic enzymes, and nucleic acid sequences encoding secretion signals, periplasm or peroxisome localization signals, or signals useful for polypeptide purification.

A circular double stranded nucleic acid molecule can be cut and thereby linearized upon treatment with restriction enzymes. An assortment of vectors, restriction enzymes, and the knowledge of the nucleotide sequences that the restriction enzymes operate upon are readily available to those skilled in the art. A nucleic acid molecule of the invention can be inserted into a vector by cutting the vector with restriction enzymes and ligating the two pieces together.

Many techniques are available to those skilled in the art to facilitate transformation or transfection of the expression construct into a prokaryotic or eukaryotic organism. These methods involve a variety of techniques, such as treating the cells with high concentrations of salt, an electric field, or detergent, to render the host cell outer  
5 membrane or wall permeable to nucleic acid molecules of interest.

In a third aspect, the invention features a nucleic acid probe for the detection of a nucleic acid encoding an hOAT polypeptide, fragment or analogue in a sample. The nucleic acid probe contains nucleotides that will hybridize specifically to a sequence of at least 14 contiguous nucleotides set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3,  
10 SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a functional derivative thereof. The probe is preferably at least 14 or more bases in length and selected to hybridize specifically to a unique region of an hOAT encoding nucleic acid.

In preferred embodiments, the nucleic acid probe hybridizes to at least 14 nucleotides of a nucleic acid encoding the full-length sequence set forth in SEQ ID NO:7,  
15 SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Under highly stringent hybridization conditions, only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 1 or 2  
20 mismatches out of 20 contiguous nucleotides.

In another preferred embodiment, the nucleic acid is an isolated conserved or unique region, for example, those useful for the design of hybridization probes to facilitate identification and cloning of additional polypeptides, or for the design of PCR probes to facilitate amplification or cloning of additional polypeptides. Such a nucleic acid may  
25 contain additional sequences in addition to the conserved or unique region, preferably 10-20 additional nucleotides on each side of the conserved or unique region, more preferably 30-40 additional nucleotides, and most preferably 75-100 additional nucleotides.

Methods for using the probes include detecting the presence or amount of hOAT polypeptide RNA in a sample by contacting the sample with a nucleic acid probe under  
30 conditions such that hybridization occurs and detecting the presence or amount of the probe bound to hOAT polypeptide RNA. The nucleic acid duplex formed between the probe and a nucleic acid sequence coding for an hOAT polypeptide may be used in the identification of the sequence of the nucleic acid detected (for example see, Nelson et al.,

in Nonisotopic DNA Probe Techniques, p. 275 Academic Press, San Diego (Kricka, ed., 1992) hereby incorporated by reference herein in its entirety, including any drawings). Kits for performing such methods may be constructed to include a container having disposed therein a nucleic acid probe.

5           Another feature of the invention is a nucleic acid molecule as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or fragments thereof, comprising one or more regions that encode an hOAT polypeptide or an hOAT domain polypeptide, where the hOAT polypeptide or the hOAT domain polypeptide is fused to a non-hOAT polypeptide or amino-terminal tag. Possible non-  
10 hOAT polypeptide or amino-terminal tag fusion partners include, for example, but are not limited to, glutathione-S-transferase (GST)-fusion proteins, green fluorescent protein (GFP) and fusions with histidine residues as described by Janknecht et al., 1991, *Proc. Natl. Sci. USA* 88:8972-8976.

          The invention also features recombinant nucleic acid, preferably in a cell or an  
15 organism. The recombinant nucleic acid may contain a sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 or a functional derivative thereof and a vector or a promoter effective to initiate transcription in a host cell. The recombinant nucleic acid can alternatively contain a transcriptional initiation region functional in a cell, a sequence complimentary to an RNA sequence  
20 encoding an hOAT polypeptide and a transcriptional termination region functional in a cell.

          Another aspect of the invention relates to a recombinant cell or tissue comprising a nucleic acid molecule encoding an hOAT polypeptide or hOAT polypeptide fragment. The recombinant cell may comprise a nucleic acid molecule encoding an hOAT  
25 polypeptide; an hOAT domain polypeptide; an hOAT polypeptide fragment or an hOAT polypeptide or polypeptide fragment fused to a non-hOAT polypeptide. The recombinant cell can harbor a nucleic acid vector that is extragenomic or intragenomic.

          Extragenomic vectors are designed with their own origins of replication allowing them to utilize the recombinant cell replication machinery to copy and propagate the  
30 vector nucleic acid sequence. These vectors are small enough that they are not likely to harbor nucleic acid sequences homologous to genomic sequences of the recombinant cell.

          Multiple intragenomic vectors are available to those skilled in the art and contain nucleic acid sequences that are homologous to nucleic acid sequences in a particular



organism's genomic DNA. These homologous sequences will result in recombination events that integrate portions of the vector into the genomic DNA. Those skilled in the art can control which nucleic acid sequences of the vector are integrated into the cell genome by flanking the portion to be incorporated into the genome with homologous sequences in the vector.

A new combination of genes or nucleic acid molecules can be introduced into an organism using a wide array of nucleic acid manipulation techniques available to those skilled in the art.

In yet another aspect, the invention features an isolated, enriched, or purified hOAT polypeptide.

It is also advantageous for some purposes that an amino acid sequence be in purified form. Purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. The substance is preferably free of contamination at a functionally significant level, for example 90%, 95%, or 99% pure. The hOAT polypeptides, or fragments thereof, of the present invention are preferably isolated, purified, or enriched from a mammal or a mammalian cell. The mammal is as defined herein and preferably is a mouse, and most preferably is a human. These polypeptides may be isolated, purified, or enriched from a cell that comprises an endogenous nucleic acid molecule that encodes the polypeptide, or from a cell that is transformed with a nucleic acid molecule that encodes the polypeptide. The polypeptide may also be chemically synthesized. Procedures for obtaining polypeptides using the above methods are well known to those skilled in the art.

The polypeptides of the invention comprise an amino acid sequence having (a) the full-length amino acid sequence encoded by the nucleic acid sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or functional derivatives thereof, or the amino acid sequences set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or functional derivatives thereof.

The invention also features an isolated, enriched, or purified hOAT polypeptide fragment.

Examples of polypeptide fragments include hOAT domains, and hOAT-specific epitopes. Such fragments may, for example, be produced by proteolytic cleavage of the full-length protein. Preferably, the fragment is obtained recombinantly by appropriately

modifying the DNA sequence encoding the proteins to delete one or more amino acids at one or more sites of the C-terminus, N-terminus, and/or within the native sequence.

Fragments of a protein can be used as antigens for the production of hOAT-specific antibodies, or used as competitors of substrates for hOAT polypeptides. It is understood that such fragments may retain one or more characterizing portions of the native complex. Examples of such retained characteristics include: transporting activity; substrate specificity; interaction with other molecules in the intact cell; regulatory functions; or binding with an antibody specific for the native complex, or an epitope thereof.

Well-known examples of domains are the SH2 (Src Homology 2) domain (Sadowski, et al., (1986) *Mol. Cell. Biol.* 6:4396; Pawson and Schlessinger, (1993) *Curr. Biol.* 3:434), the SH3 domain (Mayer, et al., (1988) *Nature* 332:272; Pawson and Schlessinger, (1993) *Curr. Biol.* 3:434), and pleckstrin (PH) domain (Ponting, (1996) *TIBS* 21:245; Haslam, et al., (1993) *Nature* 363:309), all of which are domains that mediate protein:protein interaction, and the kinase catalytic domain (Hanks and Hunter, (1995) *FASEB J* 9:576-595). The relative homology is at least 20%, more preferably at least 30% and most preferably at least 35%. Computer programs designed to detect such homologies are well known in the art.

Comparisons between the sequences of two or more polynucleotides or polypeptides can be performed using the local homology algorithm of Smith and Waterman, (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443, or the method of Pearson and Lipman, (1988) *PNAS* 85:2444. Computer programs implementing these methods can be used and include, BLAST, GAP, BESTFIT, FASTA, and TFASTA which are offered in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.

Protein motifs can be identified using computer programs readily available to those skilled in the art. One such computer program is offered by the Institute for Chemical Research at Kyoto University. The Kyoto computer program is offered at the following web site (<http://www.motif.genome.ad.jp/>).

Protein topology, such as the orientation and location of transmembrane helixes can also be identified using readily available computer programs. One such program is TopPred2 offered by Stockholm University and offered at the following web site (<http://www.biokemi.su.se/~server/toppred2/>).

Another aspect of the invention features an isolated, enriched or purified hOAT polypeptide analog.

The hOAT polypeptide analogs of the present invention preferably have a substantially similar biological activity to the proteins encoded by the full-length nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:7, or SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

A sequence that is substantially similar will preferably have at least 85% identity (more preferably at least 90% and most preferably 95-100%) to the amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

An hOAT polypeptide analog will retain some useful function such as, for example, substrate binding, transport activity, or the ability to bind to an hOAT specific antibody (as defined below). The hOAT polypeptide analog may be derived from a naturally occurring complex component by appropriately modifying the protein DNA coding sequence to add, remove, and/or to modify codons for one or more amino acids at one or more sites of the C-terminus, N-terminus, and/or within the native sequence. It is understood that such analogs having added, substituted and/or additional amino acids retain one or more characterizing portions of the native hOAT polypeptides.

Examples of hOAT polypeptides analogs include, but are not limited to, (1) chimeric proteins which comprise a portion of an hOAT polypeptide sequence fused to a non-hOAT polypeptide sequence, for example, a polypeptide sequence of glutathione-S-transferase, (2) hOAT polypeptides lacking a specific domain, for example, the protein kinase C domain, (3) epitope-tagged hOAT polypeptides or fragments for immuno detection or purification and (4) hOAT proteins having a point mutation.

Human OAT polypeptide analogs with deleted, inserted and/or substituted amino acid residues may be prepared using standard techniques well known to those of ordinary skill in the art. For example, the modified components of the analogs may be produced using site-directed mutagenesis techniques (as exemplified by Adelman et al., (1983) *DNA* 2:183 ) wherein nucleotides in the DNA coding the sequence are modified such that a modified coding sequence is produced, and thereafter expressing this recombinant DNA in

a prokaryotic or eukaryotic host cell, using techniques such as those described above. Alternatively, proteins with amino acid deletions, insertions and/or substitutions may be conveniently prepared by direct chemical synthesis, using methods well known in the art. The analogs of the hOAT polypeptides may exhibit the same qualitative biological activity  
5 as the hOAT polypeptides themselves.

In another aspect, the invention features an antibody (e.g., a monoclonal or polyclonal antibody) having specific-binding affinity to an hOAT polypeptide or hOAT polypeptide analog or fragment.

Antibodies having specific-binding affinity to an hOAT polypeptide may be used  
10 in methods for detecting the presence and/or amount of an hOAT polypeptide in a sample by contacting the sample with the antibody under conditions such that an immunocomplex forms and detecting the presence and/or amount of the antibody conjugated to the hOAT polypeptide. Diagnostic kits for performing such methods may be constructed to include a first container containing the antibody and a second container having a conjugate of a  
15 binding partner of the antibody and a label, such as, for example, a radioisotope. The diagnostic kit may also include notification of an FDA approved use and instructions therefor.

For the production of polyclonal antibodies, various host animals may be immunized by injection with the antigen. Various adjuvants may be used to increase the  
20 immunological response, depending on the host species.

Monoclonal antibodies may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. Monoclonal antibodies may be obtained by methods known to those skilled in the art. See, for example, Kohler, et al., (1975) *Nature* 256:495-497, and U.S. Patent No. 4,376,110 issued  
25 March 8, 1983, to David et al.

Still further, the invention features a hybridoma which produces an antibody having specific-binding affinity to an hOAT polypeptide.

In preferred embodiments, the hOAT antibody comprises a sequence of amino acids that is able to specifically bind an hOAT polypeptide.

30 The invention features a method for identifying human cells containing an hOAT polypeptide or a related sequence. The method involves identifying the novel polypeptide in human cells using techniques that are routine and standard in the art, such as those

described herein for identifying hOAT (*e.g.*, cloning, Southern or Northern blot analysis, Western blot analysis, immunoassay, *in situ* hybridization, PCR amplification, *etc.*).

The invention also features methods of screening cells for natural-binding partners of hOAT polypeptides. Binding partners include modulators and downstream signaling molecules such as adaptor proteins and may be identified by techniques well known in the art such as co-immunoprecipitation or by using, for example, a two-hybrid screen. (Fields and Song, U.S. Patent No. 5,283,173, issued February 1, 1994, and incorporated by reference herein). The present invention also features the purified, isolated or enriched versions of the polypeptides identified by the methods described above.

The invention provides methods for screening compounds for their ability to inhibit, or modulate the biological activity of the human organic anion transporter molecules of the invention. In preferred embodiments, cells expressing an hOAT polypeptide, including recombinant expression constructs of the invention, are contacted with a compound, and transport activity is assayed. In especially preferred embodiments, competition assays, using known human organic anion transporter substrates, such as *p*-aminohippurate, are used to test the ability of a screened compound to interfere (compete) with the uptake of a known substrate of an hOAT polypeptide. Additional preferred embodiments comprise quantitative analyses of such effects.

The present invention is also useful for the detection of substrates, analogues of substrates, inhibitors and modulators, heretofore known or unknown, for the transporters of the invention, either naturally occurring or embodied as a drug. In preferred embodiments, such substrates, analogues, or modulators may be detected in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, vitreous humor, or any other bodily fluid. In additional preferred embodiments, the invention provides methods for detecting and identifying substrates, analogues of substrates, or modulators that preferentially affect either the uptake function or the efflux function of the transporters of the invention.

One method for identifying a substance capable of modulating an hOAT polypeptide activity comprises the steps of (a) contacting an hOAT polypeptide with a test substance; and (b) determining whether the substance alters the transporting activity of said polypeptide.

Another method of identifying substances capable of modulating the function of an hOAT polypeptide comprises the following steps: (a) expressing an hOAT polypeptide in



cells; (b) adding a compound to the cells; and (c) monitoring the transporting activity of the hOAT.

A method of identifying substrates or inhibitors of hOAT polypeptides comprises the following steps: (a) adding a test substrate to a cell line expressing an hOAT polypeptide; and (b) measuring the intracellular concentration of the test substrate.

A second method of identifying substrates or inhibitors of hOAT polypeptides comprises the following steps: (a) adding a test substrate to a cell line expressing an hOAT polypeptide; (b) adding a substrate known to be transported by said hOAT polypeptides; (c) measuring whether said test substrate competes with the uptake of the known substrate.

Another method of identifying substrates of an hOAT polypeptide comprises the following steps: (a) attaching a fluorescent compound onto a test compound; (b) adding said compound to a cell line expressing an hOAT polypeptide; and (c) monitoring whether said fluorescent compound is taken into said cell line through the use of fluorescent microscopy or any other method which can detect fluorescence.

In a preferred embodiment, high-throughput screening employing 96-well plates and a microtiter® fluorescence detection system is used to detect potential modulators, substrates, analogues of substrates and inhibitors of hOAT polypeptides which can compete with binding or transport of fluorescent compounds known to bind or be transported by hOAT polypeptides.

In yet another embodiment, uptake of a test substrate by an hOAT expressing cell line is measured using knowledge of an hOAT's antiporter activity. Uptake is measured by monitoring efflux of an accompanying antiporter molecule. Efflux of the antiporter molecule can be detected using techniques known to those skilled in the art, for example, radiolabeling of the effluxed molecule and measurement using a scintillation counter.

Measurement of antiporter efflux is also amenable to high-throughput screening.

A still further aspect of the invention is the identification of substrates, analogues of substrates, inhibitors, or modulators that affect the pharmacokinetics of drugs and compounds transported by hOAT polypeptides. Substances which decrease the transporting activity of hOAT polypeptides are useful for increasing the half-life in the body of drugs and compounds excreted via human organic anion transporters.

Yet, another aspect of the invention involves substrates, and modulators, which increase the transporting activity of an hOAT. Such substances are advantageous for increasing the delivery of a drug or compound, which is transported by an hOAT, to a

target organ such as kidney or liver. For example, a modulator which increases the transporting activity of hOAT1 would increase the delivery of PAH or other drugs or compounds transported by hOAT1 to the kidney.

5 A last aspect of the invention features a cultured cell line which stably expresses an hOAT polypeptide. Such cell lines are useful for identifying substrates which are transported by hOAT polypeptides, inhibitors of hOAT polypeptides and for identifying modulators of hOAT polypeptides. Further, a panel of cells stably expressing hOAT polypeptides will be useful in studying the pharmacokinetics of various drugs and compounds. Preferably, the expression of an hOAT polypeptide in such cell lines is under  
10 the control of an inducible promoter. More preferably, said inducible promoter has a very low basal level of expression in such cell lines and high expression when induced.

A cell line stably expressing an hOAT polypeptide is useful for designing substrates which can be transported by an hOAT polypeptide at either an increased or a decreased rate. Decreasing the rate of transport can increase the effectiveness of some  
15 medicinals by making their clearance from the body occur more slowly.

Cell lines stably expressing hOAT polypeptides are also useful for the identification and development of drugs or compounds targeted to a particular organ. For example, drugs or compounds identified as substrates of hOAT1 would be good candidates for treating diseases of the kidneys whereas those identified as substrates of  
20 hOAT2 would be good candidates for treating diseases of the liver.

The OAT expressing cell lines are also useful for identifying potentially toxic compounds or drugs. For example, newly discovered or modified drugs belonging to a class of drugs known to be toxic to certain organs, e.g., kidney can be tested for transport against the panel of cells expressing human OAT1 or hOAT3 polypeptides. If the new  
25 drug or compound is transported by hOAT1 expressing cells, the new drug or compound may also be toxic to the kidney. However, if the new drug or compound, belonging to a class known to be toxic, is not transported by hOAT1 or hOAT3 expressing cells than the new drug or compound would be expected to be less toxic to the kidney than the parent class of drugs or compounds.

30 A preferred embodiment includes the identification of toxic compounds which are substrates of hOATs, and which can be used to treat diseases of organs or tissues that express hOATs. For example, toxic compounds found to be transported by hOAT1 or hOAT3 would have potential use as anti-cancer drugs to treat cancers of the kidney.

The methods of the present invention can utilize any of the molecules disclosed in the invention. These molecules include nucleic acid molecules encoding hOAT polypeptides, nucleic acid vectors, recombinant cells, polypeptides, or antibodies described herein.

- 5           The summary of the invention described above is non-limiting and other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

### **Definitions**

- 10           By "isolated" in reference to nucleic acid it is meant a polymer preferably consisting of 14 or more nucleotides conjugated to each other, including DNA or RNA that is isolated from a natural source or that is synthesized. The isolated nucleic acid of the present invention is unique in the sense that it is not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has  
15           been removed from its normal cellular (i.e., chromosomal) environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide sequence present, but that it is essentially free (about 90-95% pure at least) of nucleotide material naturally associated with it and thus is meant to be distinguished from isolated chromosomes.

- 20           By the use of the term "enriched" in reference to nucleic acid it is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2-5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a preferential reduction in the amount of other DNA or RNA present, or by a preferential  
25           increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased.

- The term "significant" here is used to indicate that the level of increase is useful to  
30           the person making such an increase, and generally means an increase relative to other nucleic acids of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no DNA or RNA from other sources. The other source DNA may, for example, comprise DNA from a yeast or bacterial genome, or a

cloning vector such as pUC19. This term distinguishes the sequence from naturally occurring enrichment events, such as viral infection, or tumor type growths, in which the level of one mRNA may be naturally increased relative to other species of mRNA. That is, the term is meant to cover only those situations in which a person has intervened to  
5 elevate the proportion of the desired nucleic acid.

The term "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/mL). The term is also chosen to  
10 distinguish clones already in existence which may encode an hOAT polypeptide but which have not been isolated from other clones in a library of clones. Thus, the term covers clones encoding an hOAT polypeptide which are isolated from other non-hOAT clones.

The term "nucleic acid molecule" describes a polymer of deoxyribonucleotides (DNA) or ribonucleotides (RNA). The nucleic acid molecule may be isolated from a  
15 natural source by cDNA cloning or subtractive hybridization or synthesized manually. The nucleic acid molecule may be synthesized manually by the triester synthetic method or by using an automated DNA synthesizer.

The term "cDNA cloning" refers to hybridizing a small nucleic acid molecule, a probe, to cDNA. The probe hybridizes (binds) to complementary sequences of cDNA.

20 The term "complementary" describes two nucleotides that can form multiple favorable interactions with one another. For example, adenine is complementary to thymine as they can form two hydrogen bonds. Similarly, guanine and cytosine are complementary since they can form three hydrogen bonds. Thus if a nucleic acid sequence contains the following sequence of bases, thymine, adenine, guanine and  
25 cytosine, a "complement" of this nucleic acid molecule would be a molecule containing adenine in the place of thymine, thymine in the place of adenine, cytosine in the place of guanine, and guanine in the place of cytosine. Because the complement can contain a nucleic acid sequence that forms optimal interactions with the parent nucleic acid molecule, such a complement can bind with high affinity to its parent molecule.

30 The term "hybridize" refers to a method of interacting a nucleic acid sequence with a DNA or RNA molecule in solution or on a solid support, such as cellulose or nitrocellulose. If a nucleic acid sequence binds to the DNA or RNA molecule with high affinity, it is said to "hybridize" to the DNA or RNA molecule. The strength of the

interaction between the probing sequence and its target can be assessed by varying the stringency of the hybridization conditions. Under highly stringent hybridization conditions, only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having one or two mismatches out of 20 contiguous nucleotides.

By "conserved nucleic acid regions", it is meant regions present on two or more nucleic acids encoding an hOAT polypeptide, to which a particular nucleic acid sequence can hybridize under lower stringency conditions. Examples of lower stringency conditions suitable for screening for nucleic acids encoding hOAT polypeptides are provided in Abe, et al. (1992) *J. Biol. Chem.* 19:13361 (hereby incorporated by reference herein in its entirety, including any drawings). Preferably, conserved regions differ by no more than 5 out of 20 contiguous nucleotides.

By "unique nucleic acid region" is meant a sequence present in a full-length nucleic acid coding for an hOAT polypeptide that is not present in a sequence coding for any other known naturally occurring polypeptide. Such regions preferably comprise 12 or more contiguous nucleotides present in the full-length nucleic acid encoding an hOAT polypeptide. In particular, a unique nucleic acid region is preferably of human origin.

The term "nucleic acid vector" relates to a single or double stranded circular nucleic acid molecule that can be transfected or transformed into cells and replicate independently or within the host cell genome.

The terms "transformation" and "transfection" refer to methods of inserting an expression construct into a cellular organism.

The term "promoter element" describes a nucleotide sequence that is incorporated into a vector that, once inside an appropriate cell, can facilitate transcription factor and/or polymerase binding and subsequent transcription of portions of the vector DNA into mRNA. The promoter element precedes the 5' end of the hOAT polypeptide nucleic acid molecule such that the latter is transcribed into mRNA. Host cell machinery then translates mRNA into a polypeptide.

By an "hOAT polypeptide" is meant the full-length amino acid sequence of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof.

The term "recombinant cell" refers to a cell that has been altered to contain a new combination of genes or nucleic acid molecules.



The term "recombinant organism" refers to an organism that has a new combination of genes or nucleic acid molecules.

The term "organism" relates to any living being comprised of at least one cell. An organism can be as simple as one eukaryotic cell or as complex as a mammal. Therefore,  
5 a recombinant organism can also be a recombinant cell, which may be a eukaryotic or a prokaryotic organism.

By "recombinant hOAT polypeptide" it is meant to include a polypeptide produced by recombinant DNA techniques such that it is distinct from a naturally occurring polypeptide either in its location (e.g., present in a different cell or tissue than found in  
10 nature), purity or structure. Generally, such a recombinant polypeptide will be present in a cell in an amount different from that normally observed in nature.

The term "eukaryote" refers to an organism comprised of cells that contain a nucleus. Eukaryotes are differentiated from "prokaryotes" which do not have a nucleus and lack other cellular structures found in eukaryotes, such as mitochondria and  
15 endoplasmic reticulum. Prokaryotes include unicellular organisms, such as bacteria, while eukaryotes are represented by yeast, invertebrates, and vertebrates.

The term "extragenomic" refers to a nucleic acid vector which does not insert into the cell genome. Thus, these vectors replicate independently of the host genome and do not recombine with or integrate into the genome.

20 The term "intragenomic" defines a nucleic acid construct that is incorporated within the cell genome.

By "isolated" in reference to a polypeptide is meant a polymer of 6, 12, 18 or more amino acids conjugated to each other, including polypeptides that are isolated from a natural source or that are synthesized. The isolated polypeptides of the present invention  
25 are unique in the sense that they are not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only amino acid chain present, but that it is essentially free (about 90-95% pure at  
30 least) of material naturally associated with it.

By the use of the term "enriched" in reference to a polypeptide it is meant that the specific amino acid sequence constitutes a significantly higher fraction (2-5 fold) of the total of amino acid sequences present in the cells or solution of interest than in normal or

diseased cells or in the cells from which the sequence was taken. This could be caused by a preferential reduction in the amount of other amino acid sequences present, or by a preferential increase in the amount of the specific amino acid sequence of interest, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other amino acid sequences present, just that the relative amount of the sequence of interest has been significantly increased.

The term "significant" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other amino acid sequences of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there are no amino acid sequences from other sources. The other source amino acid sequences may, for example, comprise amino acid sequences encoded by a yeast or bacterial genome, or a cloning vector such as pUC19. The term is meant to cover only those situations in which a person has intervened to elevate the proportion of the desired amino acid sequences.

The term "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/mL).

By "an hOAT polypeptide fragment" it is meant an amino acid sequence that is less than the full-length hOAT amino acid sequences (and which excludes the listed EST sequences) encoded by the nucleic acid sequence set forth in SEQ ID NO:1 (excluding EST clone R25797), SEQ ID NO:2 (excluding EST clone A1016020), SEQ ID NO:3 (excluding EST clone A 1016020), SEQ ID NO:4 (excluding EST clone H41333), SEQ ID NO:5, or SEQ ID NO: 6 (excluding EST clone AA705512) or the amino acid sequence (excluding the amino acids encoded by the EST clones previously listed set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12. Thus, the term "fragment" is used to indicate a polypeptide derived from the amino acid sequence of the hOAT polypeptides, of the complexes having a length less than the full-length polypeptide from which it has been derived.

By "an hOAT domain" it is meant a portion of the hOAT polypeptide having homology to amino acid sequences from one or more known proteins wherein the sequence predicts some common function, interaction or activity.

By "hOAT polypeptide analog" it is meant an amino acid sequence substantially similar to the sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:7, or SEQ ID NO:8; SEQ ID NO:9, SEQ ID  
5 NO:10, SEQ ID NO:11, or SEQ ID NO:12.

By "biological activity" it is meant an activity of the hOAT polypeptides in a cell. The biological activity of the hOAT polypeptides is related to some of the activities of the cell which include, but are not limited to, transport (uptake or excretion of a substrate from a cell), cell proliferation, mitogenesis, metastasis, tumor escape, cell adhesion,  
10 transformation, or apoptosis.

By "identity" is meant a property of sequences that measures their similarity or relationship. Identity is measured by dividing the number of identical residues in the two sequences by the total number of residues and multiplying the product by 100. Thus, two copies of exactly the same sequence have 100% identity, but sequences that are less highly  
15 conserved and have deletions, additions, or replacements have a lower degree of identity. Those skilled in the art will recognize that several computer programs are available for determining sequence identity.

An hOAT polypeptide analog may differ from the native sequence of an hOAT polypeptide in that one or more amino acids have been changed, added or deleted.  
20 Changes in amino acids may be conservative or non-conservative. By "conservative" it is meant the substitution of an amino acid for one with similar properties such as charge, hydrophobicity, structure, etc.

By "hOAT-specific epitope" it is meant a sequence of amino acids that is both antigenic and unique to hOAT.

25 By "specific-binding affinity" is meant that the antibody binds to target (hOAT) polypeptides with greater affinity than it binds to other polypeptides under specified conditions.

The term "polyclonal" refers to antibodies that are heterogenous populations of antibody molecules derived from the sera of animals immunized with an antigen or an  
30 antigenic functional derivative thereof.

"Monoclonal antibodies" are substantially homogenous populations of antibodies to a particular antigen.

The term "antibody fragment" refers to a portion of an antibody, often the hypervariable region and portions of the surrounding heavy and light chains, that displays specific binding affinity for a particular molecule. A hypervariable region is a portion of an antibody that physically binds to the polypeptide target.

5 By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example an hOAT antibody.

By "natural-binding partner" it is meant a protein, organic anion, or other molecule that interacts with an hOAT polypeptide.

10 As used herein, pharmacokinetics is the process by which a drug or compound is absorbed, distributed, metabolized and eliminated by the body.

The term "compound" includes small organic or inorganic molecules of molecular weight of preferably less than 1000 atomic units, more preferably less than 800 atomic units, and most preferably less than 500 atomic units. "Organic molecules" include all molecules that contain a carbon atom, whereas "inorganic molecules" are those that do not  
15 have a carbon atom.

The term "function" refers to the cellular role of a protein. The role of the proteins of the invention may include transport of substrates into and out of a cell, involvement in cascades controlling cell growth, migration, differentiation, gene expression, muscle contraction, glucose metabolism, cellular protein synthesis, and regulation of the cell  
20 cycle.

The term "modulates" refers to the ability of a compound to alter the function of an hOAT polypeptide. A modulator preferably activates or decreases the transporter activity of an hOAT protein. A modulator that increases the transporting activity is a positive modulator; and one that decreases the transporting activity is a negative modulator. The  
25 term "modulates" also refers to altering the function of a protein by increasing or decreasing the probability that a complex, i.e. an assembly of at least two molecules bound to one another, forms between an hOAT protein and a natural-binding partner.

The term "transporting activity," in the context of the invention, defines the ability of a transporter polypeptide to uptake a substrate into a cell or efflux a molecule out of a  
30 cell.

The term "substrate" as used herein refers to a molecule that is transported into or out of a cell by an OAT polypeptide. The substrate may be an organic compound or molecule, inorganic compound or molecule, a peptide, or a protein.

The term "activates" refers to increasing the transport or efflux of a molecule into or out of a cell.

The term "inhibitor" refers to a compound or substance that binds to a substrate-binding site and, thereby, decreases or prevents transport of an hOAT substrate.

5 The term "expressing" as used herein refers to the production of an hOAT polypeptide from a nucleic acid vector containing an hOAT gene within a cell.

The term "adding" as used herein refers to administering a solution comprising a compound to the medium bathing cells.

10 The term "functional derivative" with respect to a polypeptide is a polypeptide that possesses a biological activity (either functional or structural) or an immunological characteristic that is substantially similar to a biological activity or an immunological characteristic of a non-recombinant hOAT. A functional derivative of an hOAT polypeptide may or may not contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance  
15 of a specific function. The term "functional derivative" is intended to include the "fragments", "variants", "analogues", "homologues" or chemical derivatives of a molecule.

Similarly, a "functional derivative" of a gene encoding an hOAT polypeptide of the present invention includes "fragments", variants", or "analogues" of the gene, which  
20 may be "substantially similar" in nucleotide sequence, and which encode a molecule possessing similar activity to an hOAT polypeptide or fragment thereof. Permutations resulting from degeneracy of the genetic code are also considered functional derivatives.

A molecule is said to be "substantially similar" to another molecule if the sequence of amino acids in both molecules is substantially the same. Substantially similar amino  
25 acid molecules will possess a similar biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if one of the molecules contains additional amino acid residues not found in the other, or if the sequence of amino acid residues is not identical.

A "chemical derivative" of a polypeptide contains additional chemical moieties not  
30 normally a part of the polypeptide.

The term "mammalian" refers to such organisms as mice, rats, rabbits, goats, more preferably monkeys and apes, and most preferably humans.



**Brief Description of the Figures**

FIG. 1 shows the cDNA and deduced amino acid sequences of human OAT1.

FIG. 2 shows the cDNA and deduced amino acid sequences of human OAT2A.

FIG. 3 shows the cDNA and deduced amino acid sequences of human OAT2B.

5 FIG. 4 shows the cDNA and deduced amino acid sequences of human OAT3.

FIG. 5 shows the cDNA and deduced amino acid sequences of human OAT4.

FIG. 6 shows the cDNA and deduced amino acid sequences of human OAT5.

FIG. 7 shows the amount of  $^3\text{H}$ -PAH taken up by HeLa cells transfected with increasing amounts of hOAT1 or vector control (LacZ) cDNAs.

10 FIG. 8 shows that oocytes injected with hOAT1 RNA uptake  $^3\text{H}$ -PAH to a much greater extent than water injected oocytes.

FIG. 9 shows that ECR293 cells stably containing hOAT1, in plasmid pIND(sp1), uptake approximately 50-fold more  $^3\text{H}$ -PAH when induced by ponasterone than non-induced cells.

15 FIG. 10 shows that ECR293 cells stably containing hOAT2A, in plasmid pIND(sp1), uptake approximately 2.6-fold more  $^3\text{H}$ -PAH when induced by ponasterone than non-induced cells.

FIG. 11 shows that sulfobromophthalein but not  $\alpha$ -ketoglutarate competed with  $^3\text{H}$ -PAH uptake in ECR293/OAT2A cells induced with ponasterone.

20 FIG. 12 shows that oocytes injected with hOAT3 RNA uptake  $^3\text{H}$ -PAH to a much greater extent than water injected oocytes.

FIG. 13 (A-E) shows Northern Blot analyses of hOAT1-5 mRNA in various human tissues.

25

**DETAILED DESCRIPTION OF THE INVENTION**

30 A. **Introduction**

Organic anions are carbon-based molecules with one or more negative charges at physiological pH. They can be endogenous compounds such as bile acids or xenobiotics such as *p*-aminohippurate. Organic anion transporters are membrane proteins that

facilitate the transport of organic anions across the cell membrane. The family described herein consists of proteins sharing at least 50% amino acid sequence similarity among each other. They show broad substrate specificity, meaning that each member can transport a variety of organic anions. Hence, they are often referred to as multi-specific organic anion transporters. The mechanism of transport, though not currently elucidated, probably is a secondary or tertiary active transport involving exchange of another organic anion. An example of a tertiary transport system is the rat kidney OAT1 transporter. The primary transport system is the  $\text{Na}^+/\text{K}^+$ -ATPase, which establishes the high out-to-in  $\text{Na}^+$  gradient. This  $\text{Na}^+$  gradient then drives the cotransport of  $\text{Na}^+$  and dicarboxylates such as  $\alpha$ -ketoglutarate through a secondary  $\text{Na}^+$ /dicarboxylate transporter. Finally, the tertiary transport system is established by OAT1, which exchanges external PAH for intracellular  $\alpha$ -ketoglutarate.

The liver and kidney are two organs rich in organic anion transporters. Hepatocytes and epithelial cells of the kidney proximal tubules are polarized cells with a basolateral and apical membrane. For the hepatocyte, the basolateral membrane faces the blood-filled sinusoid. Thus, the basolateral membrane is also called the sinusoidal membrane. The apical membrane faces the canaliculus, into which bile is secreted. The hepatocyte thus can transport organic anions from the blood into the bile canaliculus across the basolateral and apical membranes. Similarly, cells of the kidney proximal tubule transport organic anions from the blood into urine across their basolateral and apical membranes. Examples of liver sinusoidal transporters are the oatp1 and oatp2, whereas OAT1 is a kidney basolateral transporter.

## **B. General Method**

The practice of the present invention employs conventional techniques of molecular and cell biology, microbiology, and recombinant DNA. Most techniques are well-described in J. Sambrook et al., "Molecular Cloning; A Laboratory Manual" (1989) and J. Celis, ed. "Cell Biology: A Laboratory Handbook" (1998).

## **30 Identification of hOAT Nucleic Acid Molecules**

Extensive searches of Genbank databases were performed using database tools available through the website maintained by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). In particular, the Basic Local Alignment

Search Tool (BLAST) was used to perform sequence similarity searches of the Genbank databases, such as the database containing human expressed sequence tags (ESTs). The EST database contains sequences of randomly selected clones from a variety of human cDNA libraries. Since each EST clone was sequenced with one primer, about 300-500 bp  
5 of sequence information is available from each clone. The BLAST programs can translate these sequences in all possible reading frames and compare them to an input protein sequence. Human ESTs showing homology to rat OAT proteins were hereby identified.

Identified EST clones can be obtained from licensed distributors of the I.M.A.G.E. Consortium Lawrence Livermore National Laboratory (LLNL) cDNA clones. The clones  
10 are then sequenced to verify their homologies to published sequences. DNA sequencing is usually done by Sanger's method of using dideoxynucleotide triphosphates in a primer extension reaction. The DNA to be sequenced, called the template, is first denatured and then annealed to a specific primer, usually between 15-25 basepairs. In one method, <sup>35</sup>S-adenosine triphosphate is incorporated into the growing DNA strand by a DNA  
15 polymerase. Materials for such a reaction are available from commercial kits such as Sequenase 2.0<sup>®</sup> from Amersham. Reaction products are separated on a long polyacrylamide gel, usually 6.0%, by electrophoresis using Tris-borate buffer. After electrophoresis, the gel is dried under vacuum and then exposed to autoradiographic film. The DNA sequence can be read from the autorad image and entered into computer for  
20 sequence analysis. Alternatively, fluorescent-tagged nucleotides can be incorporated by the polymerase chain reaction (PCR) into the template DNA using the thermal-stable polymerase from *Thermus aquaticus* (*Taq* polymerase). The PCR products can then be loaded onto an automated sequencer such as the ABI 377<sup>®</sup> (PE Applied Biosystems, Foster City, CA), which separates the products and determines the sequence.

25 Isolation of cDNA clones from a particular tissue requires the construction of a cDNA library representing messages of that tissue. Library construction entails the isolation of mRNA from the source tissue and usually a fractionation step to separate low (< 0.5 kb) and high molecular weight species. The mRNAs are then primed with a DNA oligonucleotide, either a poly-thymidine or a mixture of random primers. Reverse  
30 transcriptase is then added to synthesize the complementary DNA strand. Short DNA fragments with sticky ends, called adapters, are ligated to the cDNAs so they can be subcloned into an appropriate vector. Popular vectors include bacteriophages such as  $\lambda$ gt11 or  $\lambda$ ZAP (Stratagene, La Jolla, CA). For phage libraries, the cDNAs are ligated to

lambda phage arms at the adapter site and then packaged into phage particles. Once packaged, the library can then be amplified by infecting an appropriate bacterial host. Ready-made libraries of various human tissues are available from several commercial sources.

5           To isolate a particular gene of interest from the cDNA library, an aliquot is plated out onto large plates and then transferred to nitrocellulose or nylon membrane. The phage DNA attached to the membrane is denatured in alkali solution, neutralized, and then crosslinked to the filter by baking or UV irradiation. The filter is then hybridized with a probe of interest. The probe can be an oligonucleotide or a longer, double-stranded  
10   fragment of DNA, such as the insert of an EST clone. The double-stranded probe is usually labeled with  $^{32}\text{P}$  to high specific activity ( $>10^9$  dpm/ $\mu\text{g}$ ), denatured by boiling for 2 minutes, and added to a hybridization buffer to concentration of  $10^6$  dpm/ml. Hybridization buffers can vary in composition depending on the hybridization condition desired. One common formulation contains 0.9 M NaCl, 90 mM sodium citrate (pH 7.0),  
15   10 mM EDTA (pH 8.0), 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinylpyrrolidone, 0.1 (w/v) BSA, 0.5 % SDS, and 100  $\mu\text{g/ml}$  sheared, denatured salmon sperm DNA. Other formulations are available from commercial sources. The filters are hybridized with the probe at  $65^\circ\text{C}$  overnight or at least 16 hours. They are then washed with sodium citrate solutions with decreasing ionic strength and increasing temperature. Finally, they are  
20   exposed to autoradiographic film with an intensifying screen at  $-80^\circ\text{C}$  overnight.

          If a plaque contains DNA sequences complementary to the probe, the probe will hybridize to the complementary sequences on the filter. The plaque containing the complementary sequences is detected by exposing X-ray film to the filter. The plaque of interest can then be isolated and purified by conventional methods. Furthermore, once  
25   purified, the phage DNA can be manipulated so the relevant cDNA insert is excised from the phage into a phagmid by an *in vivo* excision procedure using appropriate host cells. The phagmids can then be amplified in bacteria and isolated by conventional methods such as alkaline lysis. Once the plasmid is purified, it can be analyzed by standard means such as restriction endonuclease or PCR mapping and DNA sequencing. DNA sequence  
30   information is analyzed for open reading frames and compared to published sequences. As discussed earlier, sets of computer programs for DNA and protein sequence analysis are commercially available.

Once a cDNA coding for a full-length transporter protein is isolated, it can be excised from its cloning vector and subcloned into an appropriate expression vector. Subcloning is a frequently used procedure in recombinant DNA technology. It involves the digestion of DNA by restriction endonucleases, fractionation of the DNA fragments by agarose gel electrophoresis, and purification of the desired fragment by a DNA-binding matrix. The purified fragment is then ligated to a precut vector of choice by adding an enzyme such as T4 DNA ligase. The new DNA construct can then be transfected into a mammalian cell line to test for functional expression. Many types of expression plasmids are commercially available and they all share common elements. An expression plasmid usually contains a bacterial origin of replication, a DNA promoter that is active in a mammalian cell line, a multiple cloning site, a transcriptional termination element, and a selective marker that confers resistance to an antibiotic. An expression plasmid harboring the cDNA of interest can be transfected into mammalian cells by several conventional means like calcium phosphate precipitation or lipofection. Many transfection kits are commercially available.

#### **Recombinant Cells Containing a Nucleic Acid Molecule Encoding an hOAT**

Cell lines which stably express hOAT polypeptides can be isolated. Such cells may be obtained by using expression vectors which carry nucleic acid sequences which encode hOAT polypeptides. The expression vectors may contain viral origins of replication and/or endogenous expression elements. Further, such expression vectors may carry nucleic acid sequences which encode for a selectable marker.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of



transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence encoding an hOAT polypeptide of the invention may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding an hOAT polypeptide of the invention, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

Two DNA sequences (such as a promoter region sequence and a sequence encoding an hOAT polypeptide of the invention) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of a gene sequence encoding an hOAT polypeptide of the invention, or (3) interfere with the ability of the gene sequence of an hOAT polypeptide of the invention to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. Thus, to express a gene encoding an hOAT polypeptide of the invention, transcriptional and translational signals recognized by an appropriate host are necessary.

The present invention encompasses the expression of a gene encoding an hOAT polypeptide of the invention (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, very efficient and convenient for the production of recombinant proteins and for the propagation of clones. Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, including other bacterial strains.

In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors may include  $\lambda$ gt10,  $\lambda$ gt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as *E. coli*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Salmonella*, *Serratia*, and the like. However, under such conditions, the polypeptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

5 To express an hOAT polypeptide of the invention (or a functional derivative thereof) in a prokaryotic cell, it is necessary to operably link the sequence encoding the hOAT polypeptide of the invention to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (*i.e.*, inducible or derepressible). Examples of constitutive promoters include the *int* promoter of  
10 bacteriophage  $\lambda$ , the *bla* promoter of the  $\beta$ -lactamase gene sequence of pBR322, and the *cat* promoter of the chloramphenicol acetyl transferase gene sequence of pPR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage  $\lambda$  ( $P_L$  and  $P_R$ ), the *trp*, *recA*, *lacZ*, *lacI*, and *gal* promoters of *E. coli*, the  $\alpha$ -amylase (Ulmanen *et al.*, (1985) *J. Bacteriol.* 162:176-182), and the  
15 promoters of the bacteriophages of *Bacillus* (Gryczan, In: The Molecular Biology of the Bacilli, Academic Press, Inc., NY, 1982), and *Streptomyces* promoters (Ward *et al.*, (1986) *Mol. Gen. Genet.* 203:468-478). Prokaryotic promoters are reviewed by Glick (1987, *Ind. Microbiol.* 1:277-282), Cenatiempo (1986, *Biochimie* 68:505-516), and Gottesman (1984, *Ann. Rev. Genet.* 18:415-442).

20 Proper expression in a prokaryotic cell also requires the presence of a ribosome-binding site upstream of the gene sequence-encoding sequence. Such ribosome-binding sites are disclosed, for example, by Gold *et al.* (1981, *Ann. Rev. Microbiol.* 35:365-404). The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein,  
25 "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant  
30 progeny have the same functionality as that of the originally transformed cell.

Host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the hOAT polypeptide of interest. Especially suitable hosts for expressing the polypeptides of

the invention are eukaryotic cells. Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, amphibian oocytes, mammalian cells either *in vivo*, or in tissue culture. Mammalian cells which may be useful as hosts include 3T3-L1 preadipocytes, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their  
5 derivatives. Preferred mammalian host cells include HeLa, EcR293, SP2/0 and J558L, as well as neuroblastoma cell lines such as IMR 332, which may provide better capacities for correct post-translational processing.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and  
10 19S, and nopaline synthase promoter and polyadenylation signal sequences. Another preferred host is an insect cell, for example the *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used (Rubin, (1988) *Science* 240:1453-1459). Alternatively, baculovirus vectors can be engineered to express large amounts of hOAT polypeptides of the invention in insect cells (Jasny, (1987) *Science*  
15 238:1653; Miller *et al.*, In: Genetic Engineering, Vol. 8, Plenum; Setlow *et al.*, eds., pp. 277-297, 1986).

Any of a series of yeast expression systems can be utilized which incorporate promoter and termination elements from the actively expressed sequences coding for glycolytic enzymes that are produced in large quantities when yeast are grown in mediums  
20 rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals. Yeast provides substantial advantages in that it can also carry out post-translational modifications. A number of recombinant DNA strategies exist utilizing strong promoter sequences and high copy number plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on  
25 cloned mammalian genes and secretes peptides bearing leader sequences (*i.e.*, pre-peptides). Several possible vector systems are available for the expression of hOAT polypeptides of the invention in a mammalian host.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational  
30 regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, cytomegalovirus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen,

myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or  
5 are subject to chemical (such as metabolite) regulation.

Expression of hOAT polypeptides of the invention in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter from the immediate-early gene of human  
10 cytomegalovirus (CMV) (Boshart M. et al., (1985) *Cell* 2:521-30, the promoter of the mouse metallothionein I gene sequence (Hamer et al., (1982) *J. Mol. Appl. Gen.* 1:273-288); the TK promoter of Herpes virus (McKnight, (1982) *Cell* 31:355-365); the SV40 early promoter (Benoist et al., (1981) *Nature* (London) 290:304-31); and the yeast gal4 gene sequence promoter (Johnston et al., (1982) *Proc. Natl. Acad. Sci. (USA)* 79:6971-  
15 6975; Silver et al., (1984) *Proc. Natl. Acad. Sci. (USA)* 81:5951-5955).

Translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes an hOAT polypeptide of the invention (or a functional derivative thereof) does not contain any intervening codons  
20 which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in the formation of a fusion protein (if the AUG codon is in the same reading frame as the hOAT polypeptide of the invention coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the hOAT polypeptide of the invention coding sequence).

25 A nucleic acid molecule encoding an hOAT polypeptide of the invention and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a nonreplicating DNA or RNA molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the  
30 transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

A vector may be employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the

introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, *e.g.*, antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama (1983, *Mol. Cell. Biol.* 3:280-289).

The introduced nucleic acid molecule can be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColEI, pSC101, pACYC 184; "Molecular Cloning: A Laboratory Manual", 1989, *supra*). *Bacillus* plasmids include pC194, pC221, pT127, and the like (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, NY, pp. 307-329, 1982). Suitable *Streptomyces* plasmids include pIJ101 (Kendall *et al.*, (1987) *J. Bacteriol.* 169:4177-4183), *Pseudomonas* plasmids are reviewed in John *et al.* (1986) *Rev. Infect. Dis.* 8:693-704), and Izaki (1978) *J. Bacteriol.* 33:729-742).

Preferred eukaryotic plasmids include, for example, pcDNA3.1 (Invitrogen), BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein *et al.*, (1982) *Miami Wntr. Symp.* 19:265-274; Broach, In: "The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470, 1981; Broach, (1982) *Cell* 28:203-204; Bollon *et al.*, (1980) *J. Clin. Hematol. Oncol.* 10:39-48; Maniatis, In: *Cell Biology: A Comprehensive Treatise*, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608, 1980).



Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, *i.e.*, transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene(s) results in the production of an hOAT polypeptide of the invention, or fragments thereof. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like). A variety of incubation conditions can be used to form the peptide of the present invention. The most preferred conditions are those which mimic physiological conditions.

Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture technique appropriate to the cell type.

Cell lines stably expressing human organic anion transporters under the control of an inducible promoter can also be isolated. For inducible expression of human organic anion transporters, the ecdysone system from Invitrogen (San Diego, CA) was used. Ecdysone is a hormone that regulates metamorphosis in insects, such as the fruitfly *Drosophila*. Like other steroid hormones, ecdysone exerts its effects by binding to a nuclear receptor, the VgR. The hormone-receptor complex then binds to a sequence of DNA called the ecdysone response element (EcRE), and activates transcription from promoters containing these elements. Since mammalian cells do not express ecdysone receptors, they normally do not respond to the insect hormone. However, if a piece of DNA under the control of an ecdysone inducible promoter is transfected into a mammalian cell line, treating the cell line with ecdysone will induce expression of the said DNA.

The cloned transporters can also be expressed in the amphibian oocytes, a widely used heterologous system for the expression of membrane proteins. Complementary RNA can be synthesized from purified cDNA *in vitro* if it is downstream of an appropriate promoter, such as the T3 or T7 phage promoter. The synthesized RNA are microinjected

into each frog oocyte, which translates the message and expresses the protein in its membranes. *Xenopus* oocytes can be obtained by surgical removal of ovaries from the frog *Xenopus laevis*. The ovary lobes are treated with collagenase to remove the vitellin layer. Oocytes can be maintained for up to two weeks in Barth solution ( 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO<sub>3</sub>, 15 mM HEPES (pH 7.6), 0.7 mM CaCl<sub>2</sub>, 0.82 mM MgSO<sub>4</sub> and 50 µg/ml gentamicin) at 16°C. Typically, about 50 nl or 10-30 ng of RNA are injected into each oocyte. The injected oocytes are maintained at 16°C for 2-3 days and then subjected to transport assays.

#### Assays for functional expression

Assaying for functional expression of transporters can be done using a labeled compound that is a known substrate of the transporter. For hOAT1, *p*-aminohippurate (PAH) is a high affinity substrate that is rapidly transported. Transfected cells or injected oocytes are washed briefly with media or Barth buffer and then incubated with the labeled PAH. After the desired incubation time, the excess label is washed away by adding cold buffer. The cells are then solubilized with SDS and the amount of label taken up can be determined by scintillation counting.

Other methods for measuring transport activity of organic anion transporters are well known in the art and are applicable to the present invention. For example, transport assays utilizing membrane vesicles was demonstrated by Yamazaki, et al., (1997) *Drug Metabolism and Disposition*, 1123-1129; and Pascolo, et al. (1998) *Biochem J.* 331:99-103.

#### Cell lines stably expressing hOAT polypeptides used to identify substrates, inhibitors and modulators of hOAT polypeptides

Cell lines stably expressing hOAT polypeptides can be used to screen for other substrates or inhibitors of hOAT polypeptides through use of a competition assay. If a test compound is well transported by an hOAT protein, it will compete with a substrate, known to be transported by a particular hOAT, for access to the transporter. Cells expressing hOAT proteins would then uptake less of the known substrate in the presence than in the absence of the competitor.

If a test compound competes with transport of a known substrate, further testing is necessary to confirm that the test compound is a substrate or inhibitor. Such confirmation can be done using techniques known to those skilled in the art, for example, the intracellular concentration of the test compound can be measured using HPLC. If a test

compound, which competes with transport of a known substrate, is also found to accumulate inside a cell, this confirms that the test substrate is a substrate and not an inhibitor of the particular hOAT being tested.

Modulators can be identified by adding a test compound to cell lines stably  
5 expressing hOAT polypeptides and measuring whether the addition of the test compound increases or decreases the transport of hOAT substrates. If a modulator is identified, which decreases or inhibits the transport of a substrate, further testing, using techniques known to those skilled in the art, is necessary to confirm that such modulators are not actually competitive inhibitors. One such technique involves adding more substrate in a  
10 competition assay to determine if said substrate can compete with the inhibitory effects of the putative modulator. If adding more substrate has no affect on the modulating activity then the modulator is not a competitive inhibitor.

#### **Nucleic Acid Probes, Methods, and Kits for Detection of the hOAT Polypeptides**

15 A nucleic acid probe of the present invention may be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain other nucleic acid molecules of the present invention. A chromosomal DNA or cDNA library may be prepared from appropriate cells according to recognized methods in the art (cf. "Molecular Cloning: A Laboratory Manual", second edition, Cold Spring Harbor Laboratory,  
20 Sambrook, Fritsch, & Maniatis, eds., 1989).

In the alternative, chemical synthesis can be carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the polypeptide of interest. The synthesized nucleic acid probes may be used as primers in a polymerase chain reaction (PCR) carried  
25 out in accordance with recognized PCR techniques, essentially according to PCR Protocols, "A Guide to Methods and Applications", Academic Press, Michael, *et al.*, eds., 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence  
30 disclosed herein using methods of computer alignment and sequence analysis known in the art ("Molecular Cloning: A Laboratory Manual", 1989, *supra*). The hybridization probes of the present invention can be labeled by standard labeling techniques such as

with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes may be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art. The nucleic acid probe may be immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The samples used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample that is compatible with the method utilized.

One method of detecting the presence of nucleic acids of the invention in a sample comprises (a) contacting the sample with the above-described nucleic acid probe under conditions such that hybridization occurs, and (b) detecting the presence of the probe bound to the nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

A kit for detecting the presence of nucleic acids of the invention in a sample comprises at least one container means having disposed therein the above-described nucleic acid probe. The kit may further comprise other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabeled probes, enzymatic labeled probes (horseradish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin). Preferably, the kit further comprises instructions for use.

### **Antibodies, Hybridomas, Methods of Use and Kits for Detection of the hOAT Polypeptides**

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The present invention relates to an antibody having binding affinity to an hOAT polypeptide of the invention. The polypeptide may have the amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof, or the amino acid sequence set forth in SEQ ID NO:7 or SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof, or at least 9 contiguous amino acids thereof (preferably, at least 20, 30, 40, 50 or 60 contiguous amino acids thereof).

The present invention also relates to an antibody having specific binding affinity to an hOAT polypeptide of the invention. Such an antibody may be isolated by comparing its binding affinity to an hOAT polypeptide of the invention with its binding affinity to other polypeptides. Those which bind selectively to an hOAT polypeptide of the invention would be chosen for use in methods requiring a distinction between an hOAT polypeptide of the invention and other polypeptides, including other polypeptides with similar amino acid sequences. Such methods could include, but should not be limited to, the analysis of altered hOAT polypeptide expression in tissue containing other polypeptides.

The hOAT polypeptides of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide could be generated as described herein and used as an immunogen. The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the present invention may be generated using one of the procedures known in the art such as chimerization or CDR grafting.

The present invention also relates to hybridomas that produce the above-described monoclonal antibodies, or binding fragment thereof. A hybridoma is an immortalized cell line that is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands, 1984; St. Groth *et al.*, (1980), *J. Immunol. Methods* 35:1-21). Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well



known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal that is immunized, the antigenicity of the polypeptide and the site of injection.

5       The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or  $\beta$ -galactosidase) or through the inclusion of an adjuvant during immunization.

10       For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Agl4 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell that produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay,  
15       western blot analysis, or radioimmunoassay (Lutz *et al.*, (1988) *Exp. Cell Res.* 175:109-124). Hybridomas secreting the desired antibodies are cloned and the class and subclass are determined using procedures known in the art (Campbell, (1984) "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", *supra*).

20       For polyclonal antibodies, antibody-containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The above-described antibodies may be detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such  
25       as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, *see* Stemberger *et al.*, (1970) *J. Histochem. Cytochem.* 18:315; Bayer *et al.*, (1979) *Meth. Enzym.* 62:308; Engval *et al.*, (1972) *Immunol.* 109:129; Goding, J. (1976) *Immunol. Meth.* 13:215). The labeled  
30       antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues that express a specific peptide.

The above-described antibodies may also be immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex

carbohydrates such as agarose and sepharose, acrylic resins such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10, 1986; Jacoby *et al.*, (1974) Meth. Enzym. 34, Academic Press, N.Y.). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as in immunochromatography.

The present invention also encompasses a method of detecting an hOAT polypeptide in a sample, comprising: (a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and (b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard ("An Introduction to Radioimmunoassay and Related Techniques" Elsevier Science Publishers, Amsterdam, The Netherlands, 1986), Bullock *et al.* ("Techniques in Immunocytochemistry," Academic Press, Orlando, FL Vol. 1, 1982; Vol. 2, 1983; Vol. 3, 1985), Tijssen ("Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands, 1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test samples used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can readily be adapted in order to obtain a sample which is testable with the system utilized.

A kit contains all the necessary reagents to carry out the previously described methods of detection. The kit may comprise: (i) a first container means containing an

above-described antibody, and (ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. Preferably, the kit also contains instructions for use. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and  
5 reagents capable of detecting the presence of bound antibodies.

Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents that are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe  
10 kits. One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats that are well known in the art.

### Examples

Example 1: Identification of human EST clones homologous to rat organic  
15 anion transporter proteins

The cloning of rat kidney organic anion transporter OAT1 (Sekine, *et al.*, (1997) *J. Biol. Chem.* 30: 18526-18529) and liver transporter OAT2 (Sekine, *et al.*, (1998) *FEBS Letters* 429: 179-182) has been previously reported. The published amino acid sequences of OAT1 and OAT2 from rat were used as input sequences to perform a BLAST search of  
20 human expressed sequence tags (EST) sequences deposited in Genbank. The BLAST search identified several sequences that show amino acid homology to rat OAT1 and OAT2.

EST clones accession Nos. R25797 and AI016020 show high homology to rat OAT1 and OAT2, respectively. The clones isolated using EST clones R25797 and  
25 A1016020 were named hOAT1 and hOAT2, respectively. The other ESTs show lower homology to rat OAT1 and OAT2. These other EST clones have been named hOAT3, hOAT4, and hOAT5. The latter three clones are not considered human homologues to the rat OAT1 and OAT2.

Example 2: Isolation of full-length human OAT1 cDNA

The human EST clone R25797, listed in Example 1, was purchased from a distributor of IMAGE Consortium (LLNL) cDNA Clones (Lennon, et al., *Genomics* 33: 151-52 (1996)). EST cDNA clone R25797 was sequenced to verify its homology to rat  
5 OAT1. The cDNA insert was excised by *NotI* and *HindIII* digestion and purified by agarose gel electrophoresis. These DNA fragments were then labeled with  $^{32}\text{P}$ - $\alpha$ -deoxyadenosine triphosphate (3000 Ci/mmol) using the Prime-a-Gene labeling system (Promega, Madison, WI) to a specific activity greater than  $1 \times 10^9$  dpm/ $\mu\text{g}$ .

A human kidney cDNA library in pTripleEx vector (Clontech, Palo Alto, CA) was  
10 plated out on 10 cm-diameter plates at a density of  $5 \times 10^4$  pfu/plate. The plates were incubated at 37°C for 6-8 hours until each plaque was about 0.3-0.5 mm in diameter. Plaque lifts were performed by placing a nylon membrane on each plate for 2 minutes. The nylon membrane was then lifted carefully from the plate and immersed in 1.5 M NaCl and 0.5 M NaOH for 2 minutes to denature the attached DNA. The nylon was then placed  
15 in 1.5 M NaCl and 0.5 M Tris (pH 8.0) for neutralization. Finally, the membrane was quickly rinsed in 2X SSC (0.3 M NaCl and 30 mM sodium citrate). The attached DNA was crosslinked by UV irradiation. Filters were incubated in hybridization buffer with radioactive probes at a concentration of  $1 \times 10^6$  dpm/ml in roller bottles at 65°C overnight. The next day, the filters were washed with 2X SSC and 0.1% SDS for 15 minutes at 25°C  
20 for two times. They were then washed with 0.2X SSC and 0.1% SDS for 1 hour at 65°C. After the final wash, filters were exposed to autoradiographic film with an intensifying screen at -80°C overnight. Positive clones, which show up as dark spots on the autorad, were identified and a 3 mm-diameter agar plug was removed from the original plate.

The positive clone was purified by subsequent rounds of hybridization until a well-  
25 isolated plaque could be obtained. The cDNA of interest was excised from the phage into a phagmid by an *in vivo* excision procedure using BM25.8 cells as host (Clontech, Palo Alto, CA). Phagmids were amplified, purified, and sequenced using standard procedures. The DNA sequence was analyzed for open reading frames and compared to published sequences.

30 One positive plaque isolated from the above procedure was 2.5 kb in length. Analysis of its sequence revealed an open-reading frame with a deduced amino acid sequence showing high homology to the rat OAT1 protein (Table 1). This clone was

designated human OAT1 (hOAT1), or the human homologue of rat OAT1. The entire cDNA and deduced amino acid sequences of human OAT1 are shown in Figure 1.

The isolated cDNA encoding for hOAT1 is predicted to encode a polypeptide with a length of 550 amino acids. Computer analysis of the deduced amino acid encoded by hOAT1 cDNA reveals consensus protein kinase C (PKC) phosphorylation sites at amino acids 271, 278, and 284.

Computer analysis using the GAP-Alignment program from the Wisconsin Genetics Software Package indicates that hOAT1 polypeptide is 88% identical to rat OAT1 polypeptide and 39% identical to rat OAT2 polypeptide (Table 1). GAP analysis also indicates that hOAT1 is 86% identical to rat OAT1 nucleotide sequence (Table 1).

EST accession number R25797 is homologous to hOAT1 cDNA at nucleotides 398-2100 (Figure 1).

**Table1**

| Homology Among Human and Rat Organic Anion Transporters |  |                 |                 |                |                |                |
|---|--|-----------------|-----------------|----------------|----------------|----------------|
|   | hOAT1<br>N <sup>1</sup> (A) <sup>2</sup> | hOAT2A<br>N (A) | hOAT2B<br>N (A) | hOAT3<br>N (A) | hOAT4<br>N (A) | hOAT5<br>N (A) |
| rat OAT1  | 86% (88%)                                | 53% (37%)       | 53% (37%)       | 60% (50%)      | 48% (36%)      | 51% (40%)      |
| rat OAT2  | 53% (39%)                                | 82% (79%)       | 82% (79%)       | 51% (37%)      | 44% (30%)      | 46% (31%)      |
| hOAT1   |  | 54% (39%)       | 55% (39%)       | 61% (51%)      | 49% (37%)      | 50% (40%)      |
| hOAT2A  |  |                 | 99% (98%)       | 52% (38%)      | 44% (32%)      | 45% (32%)      |
| hOAT2B  |  |                 |                 | 52% (39%)      | 44% (33%)      | 45% (32%)      |
| hOAT3   |  |                 |                 |                | 52% (37%)      | 52% (39%)      |
| hOAT4   |  |                 |                 |                |                | 72% (55%)      |
| hOAT5   |  |                 |                 |                |                |                |

1 (N) indicates the percentage of identical nucleotides found between compared  
nucleic  
acid sequences as determined using the GAP-Alignment program in the GCG  
software  
package, using a gap weight of 5.0 and a length weigh of 0.3.

2 (A) indicates the percentage of identical amino acids found between compared  
polypeptides as determined using the GAP-Alignment program in the GCG  
software  
package, using a gap weight of 3.0 and a length weight of 0.1.



**Example 3:** Isolation of full-length human OAT2A and OAT2B cDNA

Human OAT2 cDNA was cloned in the same manner as described for human OAT1 cDNA in Example 1 except that EST accession number-A1016020 was used as a probe. In addition, hOAT2A and hOAT2B were isolated from a cDNA library obtained from human liver rather than kidney, as was hOAT1.

The entire cDNA and deduced amino acid sequences of human OAT2A are shown in Figure 2. Human OAT2A (hOAT2A) cDNA is predicted to encode a polypeptide that is 546 amino acids in length. Computer analysis of the deduced amino acid sequence using the GAP-Alignment program indicates that human OAT2A polypeptide is 79% identical to rat OAT2 polypeptide (Table 1). GAP analysis also indicates that the nucleotide sequence for hOAT2A is 82% identical to the nucleotide sequence of rat OAT2.

EST accession number A1016020 is homologous to hOAT2A cDNA at nucleotide sequences 20-1400 (Figure 2).

The entire cDNA and deduced amino acid sequences of human OAT2B (hOAT2B) are shown in Figure 3. Human OAT2B cDNA is predicted to encode a polypeptide that is 538 amino acids in length. Human OAT2B is identical to hOAT2A except at its C-terminus end. Without wishing to be bound by any particular theory, hOAT2B could be an mRNA splice variant of hOAT2A.

**Example 4:** Isolation of full-length human OAT3 cDNA

Human OAT3 (hOAT3) cDNA was isolated as described for hOAT1 cDNA in Example 1 except that EST accession number-H41333 was used as a probe. The entire cDNA and deduced amino acid sequences of human OAT3 are shown in Figure 4.

Computer analysis of the hOAT3 cDNA sequence predicts that hOAT3 cDNA encodes a polypeptide that is 542 amino acids in length. Computer analysis of the deduced amino acid sequence reveals consensus PKC phosphorylation sites at amino acids 259, 266, 269, 511, and 527.

EST accession number H41333 is homologous to hOAT3 cDNA at nucleotides 1169-2121 (Figure 4).

**Example 5:** Isolation of full-length human OAT4 cDNA

Human OAT4 (hOAT4) cDNA was cloned in the same manner as described for human OAT1 cDNA in Example 1 except that EST accession number-AA705512 was used as a probe. In addition, hOAT4 cDNA was isolated from a cDNA library obtained from human liver rather than kidney, as was hOAT1. The entire cDNA and deduced amino acid sequences of human OAT4 are shown in Figure 5. Computer analysis of human OAT4 cDNA predicts that hOAT4 cDNA encodes a polypeptide that is 554 amino acids in length. Computer analysis of the deduced amino acid sequence reveals a consensus PKC phosphorylation site at amino acid 324.

EST accession number AA705512 is homologous to hOAT4 cDNA at nucleotides 1-1232 (Figure 5).

**Example 6:** Isolation of full-length hOAT5 cDNA

Human OAT5 (hOAT5) cDNA was cloned in the same manner as described for human OAT1 cDNA in Example 1 except that EST accession number-AAA705512 was used as a probe. In addition, hOAT5 cDNA was isolated from a cDNA library obtained from human liver rather than kidney, as was hOAT1. The entire cDNA and deduced amino acid sequences of human OAT5 are shown in Figure 6. Human OAT5 cDNA is predicted to encode a polypeptide that is 541 amino acids in length. Computer analysis of the deduced amino acid sequence reveals consensus PKC phosphorylation sites at amino acids 282, 289, 345, and 526.

EST accession number AA705512 is homologous to hOAT5 cDNA at nucleotides 1-1193 (Figure 6).

**Example 7:** Functional expression of human OAT1 in HeLa cells

Human OAT1 cDNA was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA), which contains a cytomegalovirus promoter. The plasmid construct (pcDNA-hOAT1) was then amplified and purified. The construct was transfected into HeLa cells (ATCC), cultured in 25 mm<sup>2</sup> flasks, using the Effectene transfection reagent (Qiagen, Stanford Santa Clarita, CA). Control cell lines were transfected with a pcDNA-lacZ construct. One day post-transfection, the cells were split into six (6)-well plates and cultured for another twenty-four (24) hours. Transfected cells were tested for transport of <sup>3</sup>H-*p*-aminohippurate, a classical substrate of the kidney

organic anion transporter. Since OAT1 is an antiporter, the transfected cells were first preloaded with 1 mM glutarate for one (1) hour. The glutarate-containing media was removed and replaced with media containing 50  $\mu$ M PAH at 1  $\mu$ Ci/ml. After ten (10) minutes at 37°C, the media was aspirated and the cells were washed three times with ice cold phosphate-buffered saline (PBS). The cells were then solubilized with 0.5% SDS. The amount of labeled PAH transported into the cells was determined by a scintillation counter. As shown in Fig. 7, HeLa cells transfected with hOAT1 were able to uptake PAH whereas HeLa cells transfected with a vector control were only able to uptake a very small amount of PAH (almost 10-fold less than cells transfected with a vector containing DNA encoding hOAT1).

Example 8: Functional expression of hOAT1 in *Xenopus* oocytes

Complementary hOAT1 RNA was synthesized *in vitro* from the pcDNA-hOAT1 plasmid using T7 RNA polymerase (Ambion, Austin, TX). The transcription reaction was performed at 37°C for 2 hours. The RNA was purified by phenol and chloroform extraction and precipitated with ammonium acetate and ethanol. Purified RNA was analyzed for size and purity by agarose gel electrophoresis. RNA was resuspended in water at a concentration of 0.3  $\mu$ g/ $\mu$ l. Oocytes were prepared from ovaries removed from female *Xenopus laevis* according to White et al., (1985) *PNAS* 82:4852-56. About 15 ng of hOAT1 cRNA in 50 nl was injected into each oocyte. As a control, 50 nl of water was injected into each oocyte. The injected oocytes were assayed for transport of PAH two (2) days post-injection. Oocytes were preloaded with 1 mM glutarate for 2 hours in Barth buffer. Transport assays were conducted in modified Barth buffer (100 mM NaCl, 2mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, pH 7.5) with 50  $\mu$ M PAH at 4  $\mu$ Ci/ml. After a one (1) hour incubation at 25°C, oocytes were washed with 3 ml ice cold transport assay buffer three (3) times. Each oocyte was solubilized in 0.5 ml 0.5% SDS and subjected to scintillation counting. As shown in Fig. 8, oocytes injected with the hOAT1 RNA uptake <sup>3</sup>H-PAH to a much greater extent than water injected oocytes.

Example 9: Identification of fluorescein as a substrate for hOAT1

Human embryonic kidney (HEK293) cells were transfected with pcDNA-hOAT1, as in Example 7. One day after transfection the cells were assayed for transport of fluorescein or SITS. Ten (10)  $\mu$ M of either substrate was added to the external media for

ten (10) minutes at 37°C. The cells were washed with ice-cold PBS to remove excess label and analyzed under a fluorescent microscope. Cells that take up either fluorescein or SITS are predicted to turn fluorescent green. Fluorescent microscopy revealed that hOAT1-expressing cells took up fluorescein but not SITS.

5

Example 10: Competition assay to identify substrates of hOAT1 using fluorescein

HEK293 cells were transiently transfected with pcDNA3-hOAT1 as in Example 7 and assayed for transport of fluorescein, but in the presence or absence of 0.5 mM PAH.

10 Since fluorescein and PAH are both substrates of hOAT1, they will compete for access to the transporter. As evidenced by fluorescent microscopy, hOAT1-expressing cells showed uptake of fluorescein in the absence but not in the presence of external PAH.

Example 11: Establishment of Cell lines stably expressing hOATs.

15 Vector pIND(sp1) (Invitrogen) contains the ecdysone-inducible promoter (EcRE). Using standard methods known to those in the art, cDNAs encoding hOAT1, hOAT2A, into vector pIND/sp1 downstream of its EcRE promoter. After cloning, the vectors were separately transfected into cell line EcR293 (Invitrogen). The EcR293 cell line is a derivative of cell line HEK 293 and contains a stably integrated pVgRXR vector. The  
20 pVgRXR vector constitutively expresses the ecdysone receptor and the retinoid X receptor (RXR). The ecdysone receptor and RXR form a heterodimer which, upon activation by the hormone ecdysone, binds to a promoter containing the EcRE. The pVgRXR vector was maintained within the EcR293 cell line by selection with 400 µg/ml zeocin. The pIND(sp1)-hOAT plasmids contain a neomycin resistance marker, and cells stably  
25 integrating the plasmids were selected for by culturing the cells in media supplemented with 400 µg/ml G418. After two weeks of selection, individual colonies appeared and were isolated and expanded. To test for expression of the transporter, the cells were induced with 1.25 µg/ml ponasterone, an ecdysone analogue, for twenty-four (24) hours. The cells were then subjected to standard transport assays.

30 EcR293 cells with stably integrated pIND(sp1)-hOAT1 expressed hOAT1 at a basal level that was not harmful to the cells. These cells grow at a rapid rate comparable to the parental EcR293 cells. Hence, the hOAT1 cDNA can be maintained within these cells indefinitely. Expression of hOAT1 at high levels can be easily achieved by inducing

with ponasterone for twenty-four (24) hours. As shown in Fig. 9, ECR293 cells containing hOAT1 in plasmid pIND(sp-1) uptake approximately 50-fold more PAH when induced by ponasterone than non-induced cells containing pIND(sp-1)-hOAT1. ECR293 cells stably containing hOAT2A, in plasmid pIND(sp-1), uptake approximately 2.6-fold more <sup>3</sup>H-PAH when induced by ponasterone than non-induced cells (Fig. 10). Cell lines stably expressing hOAT2B, hOAT3, hOAT4, and hOAT5 can be established using the same method as for hOAT1 and hOAT2A.

**Example 12:** Competition assay to identify new substrates of hOAT2, using a radiolabeled substrate

EcR293/OAT2A cells were assayed for transport of <sup>3</sup>H-PAH as in Example 7. The uptake of <sup>3</sup>H-PAH was blocked by 100 μM sulfobromophthalein (BSP) but not 500 μM α-ketoglutarate (AKG) (Fig. 11). The competition assay thus identified BSP and not AKG as a preferred substrate of hOAT2A.

**Example 13:** Predicting pharmacokinetics in man using a panel of hOAT expressing cells

A panel of different cell lines, each expressing a particular human OAT protein, can be used to predict pharmacokinetics for anionic drugs. For instance, PAH is a compound transported rapidly by hOAT1 and to a much lesser extent by the other human OATs disclosed in the invention. The panel of hOAT expressing cells would predict that PAH is eliminated rapidly by the human kidney, as previously described. Similarly, the competition assays in Examples 10 and 12 showed that fluorescein is a very good substrate of human OAT1 and that BSP is a good substrate of human OAT2A. Therefore, the assay would predict that fluorescein is eliminated from the human body mainly via kidney excretion and that BSP is mainly eliminated from the human body via liver excretion.

Further confirmation that a compound is a substrate of an hOAT would be done using *in vivo* tissue distribution assays. Compounds found to be transported by hOATs, using the cell line described above, would be injected into animals and followed to confirm their transport by the appropriate hOAT. For instance, a compound found to be transported by a cell line stably expressing hOAT1, would be expected to be excreted in the urine of an animal injected with the said compound. Similarly, a compound found to



be transported by a cell line expressing hOAT2, would be expected to be transported into the liver of animals injected with said compound.

Since hOAT4 and 5 have homology to OCTs, their range of substrates may include organic cations. Therefore, these transporters may be used to predict the pharmacokinetics of certain cationic drugs as well, using the methods described herein.

Example 14: Identification of hOAT substrates using antiporter activity.

EcR293/hOAT1 cells are incubated with  $^3\text{H}$ - $\alpha$ -ketoglutarate for an hour to preload with labeled  $\alpha$ -ketoglutarate. A test substrate is added to the cells. After a 10 minute incubation, an aliquot of the supernatant is removed and measured for effluxed  $\alpha$ -ketoglutarate by scintillation counting. Substrates of hOAT1 will show trans-stimulation of  $\alpha$ -ketoglutarate exchange. Inhibitors or non-substrates of hOAT1 will not stimulate  $\alpha$ -ketoglutarate efflux.

Example 15: Cytotoxicity assays using cell lines stably expressing hOAT polypeptides

EcR293 cells stably transformed with hOAT polypeptides can be used to screen for compounds potentially toxic to certain organs or tissues through use of a cytotoxicity assay. Test compounds can be added to cell lines stably expressing hOAT polypeptides. Compounds transported by hOAT polypeptides and which are toxic will cause cell death. Cell death can be measured or determined using techniques well known in the art, such as visual inspection, microscopic inspection, and dye exclusion. Using dye exclusion, healthy cells are able to exclude a particular dye whereas injured or dead cells are unable to exclude the dye.

The foregoing examples are not limiting and are merely representative of various aspects and features of the present invention. All references referred to above are incorporated herein by reference.

Example 16: Functional expression of hOAT3 in *Xenopus* oocytes

Complementary hOAT3 RNA was synthesized *in vitro* from the pcDNA-hOAT3 plasmid using T7 RNA polymerase (Ambion, Austin, TX). The transcription reaction was performed at 37°C for 2 hours. The RNA was purified by phenol and chloroform extraction and precipitated with ammonium acetate and ethanol. Purified RNA was analyzed for size and purity by agarose gel electrophoresis. RNA was resuspended in

water at a concentration of 0.5  $\mu\text{g}/\mu\text{l}$ . Oocytes were prepared from ovaries removed from female *Xenopus laevis* according to White et al., (1985) *PNAS* 82:4852-56. About 25 ng of hOAT3 cRNA in 50 nl was injected into each oocyte. As a control, 50 nl of water was injected into each oocyte. The injected oocytes were assayed for transport of PAH two (2) days post-injection. Transport assays were conducted in modified Barth buffer (100 mM NaCl, 2mM KCl, 1 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 10 mM HEPES, pH 7.5) with 50  $\mu\text{M}$  PAH at 10  $\mu\text{Ci}/\text{ml}$ . After a one (1) hour incubation at 25°C, oocytes were washed with 3 ml ice cold transport assay buffer three (3) times. Each oocyte was solubilized in 0.5 ml 0.5% SDS and subjected to scintillation counting. As shown in Fig. 12, oocytes injected with the hOAT3 RNA uptake  $^3\text{H}$ -PAH to a much greater extent than water injected oocytes.

Example 17: Determination of tissue distribution of hOATs

The individual hOAT cDNAs were labeled with  $^{32}\text{P}$ -ATP to a specific activity of  $> 1 \times 10^9$  dpm/ $\mu\text{g}$  using the Strip-EZ kit™ (Ambion). The Strip-EZ kit™ allows easy removal of probes from a blot and the blot can thus be reprobed several times without substantial loss of signal. The Human Multiple Tissue Northern blot was obtained from Clontech, which contained mRNA extracted from 12 different human tissues. The blot was successively hybridized with each hOAT probe in 5 ml of ExpressedHyb buffer (Clontech) at a final concentration of  $3\text{-}5 \times 10^6$  dpm/ml. Hybridization was carried out at 65°C for 4 hrs. The blot was washed two times with 2X SSC and 0.1% SDS at 65°C for 15 min each, and then once with 0.1X SSC and 0.1% SDS at 65°C for 1 hr. The blot was then exposed to BioMax MR autographic film (Kodak) with intensifying screen at -80°C for 24 hrs.

The northern analysis shows that hOAT1 was expressed only in human kidney (FIG 13). The OAT1 probe hybridized to a major band at about 2.4 kb. The hOAT2 probe hybridized to two major bands at about 2.4 and about 3 kb from liver and kidney, with higher levels in the liver than kidney (FIG 13). HOAT3 was expressed only in the kidney, with a major transcript of about 2.4 kb and minor transcript at about 4.3 kb (FIG 13). HOAT4 and hOAT5 were expressed exclusively in the liver (FIG 13). hOAT4 probe hybridized to a major band at about 4.3 kb and hOAT5 probe hybridized to a major band at about 2.5 kb. Hence, northern analyses indicated that hOAT1 and 3 are mainly kidney transporters and

hOAT4 and 5 are liver transporters, whereas hOAT2 is a liver and kidney transporter.

5 All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually. None of the references are admitted to be prior art.

10 One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

15 It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

20 The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and  
25 expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art,  
30 and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art

will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Other embodiments are within the following claims.

**What is claimed is:**

1. An isolated, enriched, or purified nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

5

2. An isolated, enriched, or purified nucleic acid molecule comprising a nucleic acid molecule which encodes a human OAT polypeptide selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

10

3. An isolated, enriched, or purified nucleic acid molecule which is complementary to any of the nucleic acid molecules of claims 1 and 2.

15

4. The isolated, enriched, or purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule is SEQ ID NO:2.

5. The isolated, enriched, or purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule is SEQ ID NO:3.

20

6. The isolated, enriched, or purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule is SEQ ID NO:4.

7. The isolated, enriched, or purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule is SEQ ID NO:5.

25

8. The isolated, enriched, or purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule is SEQ ID NO:6.

9. The isolated, enriched, or purified nucleic acid molecule of claim 2, wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:8.

30

10. The isolated, enriched, or purified nucleic acid molecule of claim 2, wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:9.

11. The isolated, enriched, or purified nucleic acid molecule of claim 2, wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:10.

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12. The isolated, enriched, or purified nucleic acid molecule of claim 2, wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:11.

13. The isolated, enriched, or purified nucleic acid molecule of claim 2, wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:12.

14. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence which encodes a naturally occurring variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:8, wherein the nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:2 under stringent conditions;

(b) a nucleotide sequence which encodes a naturally occurring variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:9, wherein the nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:3 under stringent conditions;

(c) a nucleotide sequence which encodes a naturally occurring variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:10, wherein the nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:4 under stringent conditions;

(d) a nucleotide sequence which encodes a naturally occurring variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:11, wherein the nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:5 under stringent conditions; and

(e) a nucleotide sequence which encodes a naturally occurring variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:12, wherein the nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:6 under stringent conditions.

15. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 83% identity to nucleotide sequence contained in SEQ ID NO:2, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

5

16. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 83% identity to nucleotide sequence contained in SEQ ID NO:3, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

10

17. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 61% identity to nucleotide sequence contained in SEQ ID NO:4, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

15

18. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 60% identity to nucleotide sequence contained in SEQ ID NO:5, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

20

19. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 60% identity to nucleotide sequence contained in SEQ ID NO:6, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

25

20. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 80% percent identical to the amino acid sequence of SEQ ID NO:8 or 9, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

30

21. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 70% percent identical to the amino acid sequence of SEQ ID NO:10, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

5

22. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 70% percent identical to the amino acid sequence of SEQ ID NO:11, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

10

23. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 70% percent identical to the amino acid sequence of SEQ ID NO:12, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

15

24. The nucleic acid molecule of claim 1 or 2, further comprising a vector or promoter effective to initiate transcription in a host cell.

25. The nucleic acid molecule of claim 24, wherein said promoter comprises an inducible promoter.

20

26. A nucleic acid probe for the detection, isolation, purification, enrichment, or amplification of a nucleic acid molecule encoding human OAT in a sample, wherein said nucleic acid probes are selected from the group of nucleic acid sequences set forth in, SEQ ID NO:2 from nucleotide 1370- 1638, SEQ ID NO: 3 from nucleotide 1370-1614, SEQ ID NO:4: from nucleotide 1-1107, SEQ ID NO: 5 from nucleotide 995-1662 and SEQ ID NO:6 from nucleotide 993-1623.

25

27. A nucleic acid probe for the detection, isolation, purification, enrichment, or amplification of a nucleic acid molecule encoding human OAT in a sample, wherein said nucleic acid probes are selected from the group of nucleic acid sequences encoding the polypeptide fragment selected from the group consisting of amino acids 132-136 of SEQ ID NO:8, amino acids 132-136 of SEQ ID NO:9, amino acids 112-116 of SEQ ID NO:10, amino acids 136-140 of SEQ ID NO:11 and amino acids 135-139 of SEQ ID NO:12.

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28. A recombinant cell comprising a nucleic acid molecule encoding a human OAT polypeptide wherein said nucleic acid molecule is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

5

29. The cell of claim 28, wherein said cell is stably transformed.

30. The cell of claim 28 or 29, wherein said cell is a mammalian cell.

10

31. The cell of claim 28 or 29, wherein said polypeptide is a fragment of the polypeptide encoded by the amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

15

32. The cell of claim 28 or 29, wherein said nucleic acid is expressed from an inducible promoter.

33. The cell of claim 32, wherein said promoter is inducible with ecdysone.

20

34. An isolated, enriched, or purified human OAT polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

25

35. The polypeptide of claim 34, wherein said polypeptide is a fragment of the polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

30

36. The polypeptide of claim 34, wherein said polypeptide is isolated, purified, or enriched from a cell that comprises an endogenous nucleic acid molecule that encodes said polypeptide.

37. The polypeptide of claim 34, wherein said polypeptide is isolated, purified, or enriched from a cell that is transformed with a nucleic acid molecule that encodes said polypeptide.

5 38. The polypeptide of claim 34, wherein said polypeptide is chemically synthesized.

39. A labeled or unlabeled antibody or antibody fragment having specific-binding affinity to a human OAT polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

40. A hybridoma which produces an antibody having specific binding affinity to a human OAT polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

20 41. A method for identifying modulators of human OAT2A, human OAT2B, human OAT3, human OAT4, or human OAT5 polypeptide, said method comprising:

- (a) contacting said hOAT polypeptide with a test substance;
- (b) adding a substrate,
- (c) measuring the activity of said hOAT polypeptide; and
- 25 (d) if said substance decreases transport, determining whether said test substance is a modulator or competitive inhibitor of said hOAT polypeptide.

42. A method for identifying a modulator of human OAT2A, human OAT2B, human OAT3, human OAT4, or human OAT5 polypeptide in a cell, said method comprising:

- (a) expressing an hOAT polypeptide in a cell;
- (b) contacting said cell with a test substance;
- (c) adding a substrate;
- (d) measuring the activity of said hOAT polypeptide; and



(e) if said substance decreases transport, determining whether said test substance is a modulator or competitive inhibitor of said hOAT.

43. A method to screen for substrates or inhibitors of human OAT2A, human OAT2B, human OAT3, human OAT4, and human OAT5 using a cell stably transformed with an hOAT nucleic acid molecule and a competition assay, said method comprising:

(a) adding a test substrate to a cell line expressing said human OAT polypeptides;

(b) adding a substrate known to be transported by said human OAT polypeptides in said cell line;

(c) measuring the ability of said test substrate to compete with the uptake of said substrate known to be transported by human OAT1, human OAT2A, human OAT2B, human OAT3, human OAT4, and human OAT5; and

(d) confirming whether said test substrate is an inhibitor or substrate.

44. The method of claim 43, wherein said test substrate and/or known substrate are fluorescent or conjugated with a fluorescent labeled.

45. The method of claim 44, wherein said assay is evaluated using high-throughput screening.

46. A method for identifying substrates of human OAT polypeptides, said method comprising:

(a) loading a cell line expressing an hOAT polypeptide with a substance known to be effluxed from the interior of said cell as part of the antiporter activity of said hOAT polypeptide

(b) adding a test substrate to said cell line;

(c) measuring the efflux of said antiporter substance; and

(d) determining if said test substrate affected the efflux of said antiporter substance.

47. The cell of claim 28 or 29, wherein said cell is a yeast cell.

48. The cell of claim 28 or 29, wherein said cell is an insect cell.

49. A method of preparing an hOAT polypeptide comprising:
- (a) culturing the recombinant cell of claim 29 under conditions that permit expression of the hOAT polypeptide; and
  - (b) isolating said polypeptide.

5

50. An amphibian oocyte containing a nucleic acid molecule encoding for a human OAT polypeptide according to claim 2.

51. The amphibian oocyte of claim 50 that is a *Xenopus laevis* oocyte.

10

52. The nucleic acid molecules of claim 1 or 2, wherein said nucleic acid molecules comprise one or more regions that encode an hOAT polypeptide or an hOAT polypeptide fragment, where the hOAT polypeptide or the hOAT polypeptide fragment is fused to a non-hOAT polypeptide or epitope tag.

15

53. The nucleic acid molecule of claim 52, wherein said non-hOAT polypeptide or epitope tag is selected from the group consisting of glutathione-S-transferase, green-fluorescent protein and an amino terminal tag composed of histidine residues.

20

54. A method to identify toxic compounds transported by hOAT polypeptides comprising:

- (a) expressing an hOAT polypeptide in cells;
- (b) adding a test substrate to said cells; and
- (c) determining if said cells are viable.

25

CTGAGCTGACCTGACCCCCAAAGTGAAGGAGAAGCTGCAAGGGAAAAGGGAGGGACAGAT  
1 -----+-----+-----+-----+-----+-----+-----+ 60  
GACTCGACTGGACTGGGGGTTTCACTTCCTCTTCGACGTTCCCTTTTCCCTCCCTGTCTA  
CAGGGAGACCGGGGAAGAAGGAGGAGCAGCCAAGGAGGCTGCTGTCCCCCCACAGAGCAG  
61 -----+-----+-----+-----+-----+-----+-----+ 120  
GTCCCTCTGGCCCCCTTCTTCCTCCTCGTCGGTTCCTCCGACGACAGGGGGGTGTCTCGTC  
CTCGGACTCAGCTCCCGGAGCAACCCAGCTGCGGAGGCAACGGCAGTGCTGCTCCTCCAG  
121 -----+-----+-----+-----+-----+-----+-----+ 180  
GAGCCTGAGTCGAGGGCCTCGTTGGGTGCGACGCCTCCGTTGCCGTCACGACGAGGAGGTC  
CGAAGGACAGCAGGCAGGCAGACAGACAGAGGTCTGGGACTGGAAGGCCTCAGCCCCCA  
181 -----+-----+-----+-----+-----+-----+-----+ 240  
GCTTCCTGTCGTCCGTCCGTCTGTCTGTCTCCAGGACCCTGACCTTCCGGAGTCGGGGGT  
GCCACTGGGCTGGGCCTGGCCCAATGGCCTTTAATGACCTCCTGCAGCAGGTGGGGGGTG  
241 -----+-----+-----+-----+-----+-----+-----+ 300  
CGGTGACCCGACCCGGACCGGGTTACCGGAAATTAAGGAGGACGTCGTCCACCCCCCAC  
M A F N D L L Q Q V G G V -  
TCGGCCGCTTCCAGCAGATCCAGGTCACCCTGGTGGTCTCCCCCTGCTCCTGATGGCTT  
301 -----+-----+-----+-----+-----+-----+-----+ 360  
AGCCGGCGAAGGTCGTCTAGGTCCAGTGGGACCACCAGGAGGGGGACGAGGACTACCGAA  
G R F Q Q I Q V T L V V L P L L L M A S -  
CTCACAACACCCTGCAGAACTTCACTGCTGCCATCCCTACCCACCACTGCCGCCCCGCTG  
361 -----+-----+-----+-----+-----+-----+-----+ 420  
GAGTGTGTGGGACGTCTTGAAGTGACGACGGTAGGGATGGGTGGTGACGGCGGGCGGAC  
H N T L Q N F T A A I P T H H C R P P A -  
CCGATGCCAACCTCAGCAAGAACGGGGGGCTGGAGGTCTGGCTGCCCCGGGACAGGCAGG  
421 -----+-----+-----+-----+-----+-----+-----+ 480  
GGCTACGGTTGGAGTCGTTCTTGCCCCCGACCTCCAGACCGACGGGGCCCTGTCCGTCC  
D A N L S K N G G L E V W L P R D R Q G -  
GGCAGCCTGAGTCCTGCCTCCGCTTCACCTCCCCGCAGTGGGGACTGCCCTTTCTCAATG  
481 -----+-----+-----+-----+-----+-----+-----+ 540  
CCGTCCGACTCAGGACGGAGGCGAAGTGGAGGGGCGTCACCCCTGACGGGAAAGAGTTAC  
Q P E S C L R F T S P Q W G L P F L N G -  
GCACAGAAGCCAATGGCACAGGGGGCCACAGAGCCCTGCACCGATGGCTGGATCTATGACA  
541 -----+-----+-----+-----+-----+-----+-----+ 600  
CGTGTCTTCGGTTACCGTGTCCCCGGTGTCTCGGGACGTGGCTACCGACCTAGATACTGT  
T E A N G T G A T E P C T D G W I Y D N -  
ACAGCACCTTCCCATCTACCATCGTGACTGAGTGGGACCTTGTGTGCTCTCACAGGGCCC  
601 -----+-----+-----+-----+-----+-----+-----+ 660

FIGURE 1  
Sheet 1 of 4

TGTCGTGGAAGGGTAGATGGTAGCACTGACTCACCTGGAACACACGAGAGTGTCCCGGG  
S T F P S T I V T E W D L V C S H R A L -  
TACGCCAGCTGGGCCAGTCCTTGTACATGGTGGGGGTGCTGCTCGGAGCCATGGTGTTCG  
661 -----+-----+-----+-----+-----+-----+-----+ 720  
ATGCGGTGCGACCGGGTCAGGAACATGTACCACCCCCACGACGAGCCTCGGTACCACAAGC  
R Q L A Q S L Y M V G V L L G A M V F G -  
GCTACCTTGCAGACAGGCTAGGCCGCCGGAAGGTACTCATCTTGAACCTACCTGCAGACAG  
721 -----+-----+-----+-----+-----+-----+-----+ 780  
CGATGGAACGTCTGTCCGATCCGGCGGCCTTCCATGAGTAGAACTTGATGGACGTCTGTC  
Y L A D R L G R R K V L I L N Y L Q T A -  
CTGTGTCAGGGACCTGCGCAGCCTTCGCACCCAACTTCCCCATCTACTGCGCCTTCCGGC  
781 -----+-----+-----+-----+-----+-----+-----+ 840  
GACACAGTCCCTGGACGCGTCGGAAGCGTGGGTTGAAGGGGTAGATGACGCGGAAGGCCG  
V S G T C A A F A P N F P I Y C A F R L -  
TCCTCTCGGGCATGGCTCTGGCTGGCATCTCCCTCAACTGCATGACACTGAATGTGGAGT  
841 -----+-----+-----+-----+-----+-----+-----+ 900  
AGGAGAGCCCGTACCGAGACCGACCGTAGAGGGAGTTGACGTACTGTGACTTACACCTCA  
L S G M A L A G I S L N C M T L N V E W -  
GGATGCCCATTACACACGGGCCTGCGTGGGCACCTTGATTGGCTATGTCTACAGCCTGG  
901 -----+-----+-----+-----+-----+-----+-----+ 960  
CCTACGGGTAAGTGTGTGCCCCGGACGCACCCGTGGAATAACCGATACAGATGTTCGGACC  
M P I H T R A C V G T L I G Y V Y S L G -  
GCCAGTTCCTCCTGGCTGGTGTGGCCTACGCTGTGCCCCACTGGCGCCACCTGCAGCTAC  
961 -----+-----+-----+-----+-----+-----+-----+ 1020  
CGGTCAAGGAGGACCGACCACACCGGATGCGACACGGGGTGACCGCGGTGGACGTTCGATG  
Q F L L A G V A Y A V P H W R H L Q L L -  
TGGTCTCTGCGCCTTTTTTTCCTTCTTCATCTACTCCTGGTTCTTCATTGAGTCGGCCC  
1021 -----+-----+-----+-----+-----+-----+-----+ 1080  
ACCAGAGACGCGGAAAAAACGGAAGAAGTAGATGAGGACCAAGAAGTAACTCAGCCGGG  
V S A P F F A F F I Y S W F F I E S A R -  
GCTGGCACTCCTCCTCCGGGAGGCTGGACCTCACCTGAGGGCCCTGCAGAGAGTCGCCC  
1081 -----+-----+-----+-----+-----+-----+-----+ 1140  
CGACCGTGAGGAGGAGGCCCTCCGACCTGGAGTGGGACTCCCGGGACGTCTCTCAGCGGG  
W H S S S G R L D L T L R A L Q R V A R -  
GGATCAATGGGAAGCGGGAAGAAGGAGCCAAATTGAGTATGGAGGTACTCCGGGCCAGTC  
1141 -----+-----+-----+-----+-----+-----+-----+ 1200

FIGURE 1  
Sheet 2 of 4

CCTAGTTACCCTTCGCCCTTCTTCCTCGGTTTAACTCATACCTCCATGAGGCCCGGTCAG  
I N G K R E E G A K L S M E V L R A S L -  
TGCAGAAGGAGCTGACCATGGGCAAAGGCCAGGCATCGGCCATGGAGCTGCTGCGCTGCC  
1201 -----+-----+-----+-----+-----+-----+ 1260  
ACGTCTTCCTCGACTGGTACCCGTTTCCGGTCCGTAGCCGGTACCTCGACGACGCGACGG  
Q K E L T M G K G Q A S A M E L L R C P -  
CCACCCTCCGCCACCTCTTCCTCTGCCTCTCCATGCTGTGGTTTGCCACTAGCTTTGCAT  
1261 -----+-----+-----+-----+-----+-----+ 1320  
GGTGGGAGGCGGTGGAGAAGGAGACGGAGAGGTACGACACCAAACGGTGATCGAAACGTA  
T L R H L F L C L S M L W F A T S F A Y -  
ACTATGGGCTGGTCATGGACCTGCAGGGCTTTGGAGTCAGCATCTACCTAATCCAGGTGA  
1321 -----+-----+-----+-----+-----+-----+ 1380  
TGATACCCGACCAGTACCTGGACGTCCCGAAACCTCAGTCGTAGATGGATTAGGTCCACT  
Y G L V M D L Q G F G V S I Y L I Q V I -  
TCTTTGGTGCTGTGGACCTGCCTGCCAAGCTTGTGGGCTTCCTTGTCATCAACTCCCTGG  
1381 -----+-----+-----+-----+-----+-----+ 1440  
AGAAACCACGACACCTGGACGGACGGTTCGAACACCCGAAGGAACAGTAGTTGAGGGACC  
F G A V D L P A K L V G F L V I N S L G -  
GTCGCCGGCCTGCCCAGATGGCTGCACTGCTGCTGGCAGGCATCTGCATCCTGCTCAATG  
1441 -----+-----+-----+-----+-----+-----+ 1500  
CAGCGGCCGGACGGGTCTACCGACGTGACGACGACCGTCCGTAGACGTAGGACGAGTTAC  
R R P A Q M A A L L L A G I C I L L N G -  
GGGTGATACCCAGGACCAGTCCATTGTCCGAACCTCTCTTGCTGTGCTGGGGAAGGGTT  
1501 -----+-----+-----+-----+-----+-----+ 1560  
CCCACTATGGGGTCTTGGTCAGGTAACAGGCTTGGAGAGAACGACACGACCCCTTCCCAA  
V I P Q D Q S I V R T S L A V L G K G C -  
GTCTGGCTGCCTCCTTCAACTGCATCTTCCTGTATACTGGGGAAGTGTATCCCACAATGA  
1561 -----+-----+-----+-----+-----+-----+ 1620  
CAGACCGACGGAGGAAGTTGACGTAGAAGGACATATGACCCCTTGACATAGGGTGTTACT  
L A A S F N C I F L Y T G E L Y P T M I -  
TCCGGCAGACAGGCATGGGAATGGGCAGCACCATGGCCCGAGTGGGCAGCATCGTGAGCC  
1621 -----+-----+-----+-----+-----+-----+ 1680  
AGGCCGTCTGTCCGTACCCTTACCCGTCGTGGTACCGGGCTCACCCGTCGTAGCACTCGG  
R Q T G M G M G S T M A R V G S I V S P -  
CACTGGTGAGCATGACTGCCGAGCTCTACCCCTCCATGCCTCTCTTCATCTACGGTGCTG  
1681 -----+-----+-----+-----+-----+-----+ 1740

FIGURE 1  
Sheet 3 of 4



GTGACCACTCGTACTGACGGCTCGAGATGGGGAGGTACGGAGAGAAGTAGATGCCACGAC  
L V S M T A E L Y P S M P L F I Y G A V -  
TTCCTGTGGCCGCCAGCGCTGTCACTGTCCTCCTGCCAGAGACCCTGGGCCAGCCACTGC  
1741 -----+-----+-----+-----+-----+-----+ 1800  
AAGGACACCGGCGGTCGCGACAGTGACAGGAGGACGGTCTCTGGGACCCGGTCGGTGACG  
P V A A S A V T V L L P E T L G Q P L P -  
CAGACACGGTGCAGGACCTGGAGAGCAGGAAAGGGAAACAGACGCGACAGCAACAAGAGC  
1801 -----+-----+-----+-----+-----+-----+ 1860  
GTCTGTGCCACGTCCTGGACCTCTCGTCCTTTCCCTTTGTCTGCGCTGTCGTTGTTCTCG  
D T V Q D L E S R K G K Q T R Q Q Q E H -  
ACCAGAAGTATATGGTCCCCTGCAGGCCTCAGCACAAGAGAAGAATGGACTCTGAGGAC  
1861 -----+-----+-----+-----+-----+-----+ 1920  
TGGTCTTCATATAACCAGGGTGACGTCCGGAGTCGTGTTCTCTTCTTACCTGAGACTCCTG  
Q K Y M V P L Q A S A Q E K N G L \*  
TGAGAAGGGGCCTTACAGAACCCTAAAGGGAGGGAAGGTCCTACAGGTCTCCGGCCACCC  
1921 -----+-----+-----+-----+-----+-----+ 1980  
ACTCTTCCCCGGAATGTCTTGGGATTTCCCTCCCTTCCAGGATGTCCAGAGGCCGGTGGG  
ACACAAGGAGGAGGAAGAGGAAATGGTGACCCAAGTGTGGGGGTTGTGGTTCAGGAAAGC  
1981 -----+-----+-----+-----+-----+-----+ 2040  
TGTGTTCTCCTCCTTCTCCTTTACCACTGGGTTCACACCCCCAACACCAAGTCCTTTTCG  
ATCTTCCCAGGGGTCCACCTCCCTTTATAAACCCCAACCAGAACCACATCATTAAGGTT  
2041 -----+-----+-----+-----+-----+-----+ 2100  
TAGAAGGGTCCCCAGGTGGAGGGAAATATTTGGGGTGGTCTTGGTGTAGTAATTTTCCAA  
TGACTGCGCACCAAAAAAAAAAAAAAAAAA  
2101 -----+-----+-----+-----+-----+ 2127  
ACTGACGCGTGGTTTTTTTTTTTTTTTT

FIGURE 1  
Sheet 4 of 4

CTGCACCTGAAGCATTGTTGGTGGGTGAGCAGCATGGGCTTTGAGGAGCTGCTGGAGCAGGT  
1 -----+-----+-----+-----+-----+-----+-----+ 60  
GACGTGGACTTCGTAAACCACCCACTCGTCGTACCCGAAACTCCTCGACGACCTCGTCCA  
M G F E E L L E Q V -  
GGGCGGCTTTGGGCCCTTCCAACCTGCGGAATGTGGCACTGCTGGCCCTGCCCCGAGTGCT  
61 -----+-----+-----+-----+-----+-----+-----+ 120  
CCCGCCGAAACCCGGGAAGGTTGACGCCTTACACCGTGACGACCGGGACGGGGCTCACGA  
G G F G P F Q L R N V A L L A L P R V L -  
GCTACCACTGCACTTCCTCCTGCCCATCTTCCTGGCTGCCGTGCCCTGCCACCGATGTGC  
121 -----+-----+-----+-----+-----+-----+-----+ 180  
CGATGGTGACGTGAAGGAGGACGGGTAGAAGACCGACGGCACGGACGGGTGGCTACACG  
L P L H F L L P I F L A A V P A H R C A -  
CCTGCCGGGTGCCCCCTGCCAACTTCAGCCATCAGGATGTGTGGCTGGAGGCCCATCTTCC  
181 -----+-----+-----+-----+-----+-----+-----+ 240  
GGACGGCCACGGGGACGGTTGAAGTCGGTAGTCCTACACACCGACCTCCGGGTAGAAGG  
L P G A P A N F S H Q D V W L E A H L P -  
CCGGGAGCCTGATGGCACGCTCAGCTCCTGCCTCCGCTTTGCCTATCCCCAGGCTCTCCC  
241 -----+-----+-----+-----+-----+-----+-----+ 300  
GGCCCTCGGACTACCGTGCGAGTCGAGGACGGAGGCGAAACGGATAGGGGTCCGAGAGGG  
R E P D G T L S S C L R F A Y P Q A L P -  
CAACACCACGTTGGGGGAAGAAAGGCAGAGCCGTGGGGAGCTGGAGGATGAACCTGCCAC  
301 -----+-----+-----+-----+-----+-----+-----+ 360  
GTTGTGGTGCAACCCCTTCTTTCCGTCTCGGCACCCCTCGACCTCCTACTTGGACGGTG  
N T T L G E E R Q S R G E L E D E P A T -  
AGTGCCCTGCTCTCAGGGCTGGGAGTACGACCACTCAGAATTCTCCTCTACCATTGCAAC  
361 -----+-----+-----+-----+-----+-----+-----+ 420  
TCACGGGACGAGAGTCCCGACCCTCATGCTGGTGAGTCTTAAGAGGAGATGGTAACGTTG  
V P C S Q G W E Y D H S E F S S T I A T -  
TGAGTGGGATCTGGTGTGTGAGCAGAAAGGTCTGAACAGAGCTGCGTCCACTTTCTTCTT  
421 -----+-----+-----+-----+-----+-----+-----+ 480  
ACTCACCTAGACCACACACTCGTCTTTCCAGACTTGTCTCGACGCAGGTGAAAGAAGAA  
E W D L V C E Q K G L N R A A S T F F F -  
CGCCGGTGTGCTGGTGGGGGCTGTGGCCTTTGGATATCTGTCCGACAGGTTTGGGCGGCG  
481 -----+-----+-----+-----+-----+-----+-----+ 540  
GCGGCCACACGACCACCCCGACACCGGAAACCTATAGACAGGCTGTCCAAACCCGCCGC  
A G V L V G A V A F G Y L S D R F G R R -

FIGURE 2

Sheet 1 of 5

541 GCGTCTGCTGCTGGTAGCCTACGTGAGTACCCTGGTGCTGGGCCTGGCATCTGCAGCCTC  
-----+-----+-----+-----+-----+-----+ 600  
CGCAGACGACGACCATCGGATGCACTCATGGGACCACGACCCGGACCGTAGACGTCCGAG  
R L L L V A Y V S T L V L G L A S A A S -  
CGTCAGCTATGTAATGTTTGCCATCACCCGCACCCTTACTGGCTCAGCCCTGGCTGGTTT  
601 -----+-----+-----+-----+-----+-----+ 660  
GCAGTCGATACATTACAAACGGTAGTGGGCGTGGAATGACCGAGTCGGGACCGACCAAA  
V S Y V M F A I T R T L T G S A L A G F -  
TACCATCATCGTGATGCCACTGGAGCTGGAGTGGCTGGATGTGGAGCACCGCACCGTGGC  
661 -----+-----+-----+-----+-----+-----+ 720  
ATGGTAGTAGCACTACGGTGACCTCGACCTCACCGACCTACACCTCGTGGCGTGGCACCG  
T I I V M P L E L E W L D V E H R T V A -  
TGGAGTCCTGAGCAGCACCTTCTGGACAGGGGGCGTGATGCTGCTGGCACTGGTTGGGTA  
721 -----+-----+-----+-----+-----+-----+ 780  
ACCTCAGGACTCGTCGTGGAAGACCTGTCCCCCGCACTACGACGACCGTGACCAACCCAT  
G V L S S T F W T G G V M L L A L V G Y -  
CCTGATACGGGACTGGCGATGGCTTCTGCTAGCTGTCACCCTGCCTTGTGCCCCAAGCAT  
781 -----+-----+-----+-----+-----+-----+ 840  
GGACTATGCCCTGACCGCTACCGAAGACGATCGACAGTGGGACGGAACACGGGGTTCGTA  
L I R D W R W L L L A V T L P C A P S I -  
CCTCAGCCTCTGGTGGGTGCCTGAGTCTGCACGCTGGCTTCTGACCCAAGGCCATGTGAA  
841 -----+-----+-----+-----+-----+-----+ 900  
GGAGTCGGAGACCACCCACGGACTCAGACGTGCGACCGAAGACTGGGTTCGGGTACACTT  
L S L W W V P E S A R W L L T Q G H V K -  
AGAGGCCACAGGTACTTGCTCCACTGTGCCAGGCTCAATGGGCGGCCAGTGTGTGAGGA  
901 -----+-----+-----+-----+-----+-----+ 960  
TCTCCGGGTGTCCATGAACGAGGTGACACGGTCCGAGTTACCCGCCGGTCACACACTCCT  
E A H R Y L L H C A R L N G R P V C E D -  
CAGCTTCAGCCAGGAGGCTGTGAGCAAAGTGGCCGCCGGGGAACGGGTGGTCCGAAGACC  
961 -----+-----+-----+-----+-----+-----+ 1020  
GTCGAAGTCGGTCCTCCGACACTCGTTTCACCGGCGGCCCTTGCCCACCAGGCTTCTGG  
S F S Q E A V S K V A A G E R V V R R P -  
TTCATACCTAGACCTGTTCCGCACACCACGGCTCCGACACATCTCACTGTGCTGCGTGGT  
1021 -----+-----+-----+-----+-----+-----+ 1080  
AAGTATGGATCTGGACAAGGCGTGTGGTGCCGAGGCTGTGTAGAGTGACACGACGCACCA  
S Y L D L F R T P R L R H I S L C C V V -

FIGURE 2

Sheet 2 of 5

GGTGTGGTTCGGAGTGAACCTTCTCCTATTACGGCCTGAGTCTGGATGTGTCTGGGGCTGGG  
1081 -----+-----+-----+-----+-----+-----+-----+ 1140  
CCACACCAAGCCTCACTTGAAGAGGATAATGCCGGACTCAGACCTACACAGCCCCGACCC  
V W F G V N F S Y Y G L S L D V S G L G -  
GCTGAACGTGTACCAGACACAGCTGTTGTTCTGGGGCTGTGGAAGTGCCTTCCAAGCTGCT  
1141 -----+-----+-----+-----+-----+-----+-----+ 1200  
CGACTTGCACATGGTCTGTGTCTGACAACAAGCCCCGACACCTTGACGGGAGGTTCGACGA  
L N V Y Q T Q L L F G A V E L P S K L L -  
GGTCTACTTGTCTGGTTCGCTACGCAGGACGCCGCCTCACGCAAGCCGGGACACTGCTGGG  
1201 -----+-----+-----+-----+-----+-----+-----+ 1260  
CCAGATGAACAGCCACGCGATGCGTCCTGCGGCGGAGTGCGTTCGGCCCTGTGACGACCC  
V Y L S V R Y A G R R L T Q A G T L L G -  
CACGGCCCTGGCGTTCGGCACTAGACTGCTAGTGTCTCCGATATGAAGTCCTGGAGCAC  
1261 -----+-----+-----+-----+-----+-----+-----+ 1320  
GTGCCGGGACCGCAAGCCGTGATCTGACGATCACAGGAGGCTATACTTCAGGACCTCGTG  
T A L A F G T R L L V S S D M K S W S T -  
TGTCCTGGCAGTGATGGGGAAAGCTTTTCTGAAGCTGCCTTCACCACTGCCTACCTGTT  
1321 -----+-----+-----+-----+-----+-----+-----+ 1380  
ACAGGACCGTCACTACCCCTTTCGAAAAGACTTCGACGGAAGTGGTGACGGATGGACAA  
V L A V M G K A F S E A A F T T A Y L F -  
CACTTCAGAGTTGTACCCTACGGTGCTCAGACAGACAGGGATGGGGCTGACTGCACTGGT  
1381 -----+-----+-----+-----+-----+-----+-----+ 1440  
GTGAAGTCTCAACATGGGATGCCACGAGTCTGTCTGTCCCTACCCGACTGACGTGACCA  
T S E L Y P T V L R Q T G M G L T A L V -  
GGGCCGGCTGGGGGGCTCTTTGGCCCCACTGGCGGCCTTGCTGGATGGAGTGTGGCTGTC  
1441 -----+-----+-----+-----+-----+-----+-----+ 1500  
CCCGGCCGACCCCCCGAGAAACCGGGGTGACCGCCGGAACGACCTACCTCACACCGACAG  
G R L G G S L A P L A A L L D G V W L S -  
ACTGCCCAAGCTTACTTATGGGGGGATCGCCCTGCTGGCTGCCGGCACCGCCCTCCTGCT  
1501 -----+-----+-----+-----+-----+-----+-----+ 1560  
TGACGGGTTCGAATGAATACCCCCCTAGCGGGACGACCGACGGCCGTGGCGGGAGGACGA  
L P K L T Y G G I A L L A A G T A L L L -  
GCCAGAGACGAGGCAGGCACAGCTGCCAGAGACCATCCAGGACGTGGAGAGAAAGAGTGC  
1561 -----+-----+-----+-----+-----+-----+-----+ 1620  
CGGTCTCTGCTCCGTCCGTGTCGACGGTCTCTGGTAGGTCTGACCTCTCTTTCTCACG  
P E T R Q A Q L P E T I Q D V E R K S A -

FIGURE 2

Sheet 3 of 5

CCCAACCAGTCTTCAGGAGGAAGAGATGCCCATGAAGCAGGTCCAGAACTAAGTGGGAGT  
1621 -----+-----+-----+-----+-----+-----+ 1680  
GGGTTGGTCAGAAGTCCTCCTTCTCTACGGGTACTTCGTCCAGGTCTTGATTCACCTCA  
  
P T S L Q E E E M P M K Q V Q N \*  
  
GGAGGCAGGCCCTCCACAGAAGCTCTGCAGCAGGGGCTGGGAGAGCAGAAGGGCAGGCCC  
1681 -----+-----+-----+-----+-----+-----+ 1740  
CCTCCGTCCGGGAGGTGTCTTCGAGACGTCGTCCCCGACCCTCTCGTCTTCCCGTCCGGG  
  
TGCAACTCAGGCTGGGAGTATCGAACCCTCTGCCTAGGGCCGGAGTTGCTGCCAGTACCC  
1741 -----+-----+-----+-----+-----+-----+ 1800  
ACGTTGAGTCCGACCCTCATAGCTTGGGAGACGGATCCCGGCCCTCAACGACGGTCATGGG  
  
GCTCCCTCTGCTCATCCATCCTTGATTATTTGGCTTCTAGGAACAGTTGACTTCCCAGAA  
1801 -----+-----+-----+-----+-----+-----+ 1860  
CGAGGGAGACGAGTAGGTAGGAATAATAAACCGAAGATCCTTGTCAACTGAAGGGTCTT  
  
TGCAGTGGGCTGCTGGGCACCCCTCTCACGGTTGGGGAGGATTCTGTAAATAAAGGTGCC  
1861 -----+-----+-----+-----+-----+-----+ 1920  
ACGTCACCCGACGACCCGTGGGGAGAGTGCCAACCCCTCCTAAGACATTTATTTCCACGG  
  
CCTTGGGTGGGGCAATGGTGACGAGCTGTGGGAAGAGCCCTGGATAGGAAGCCACTGAG  
1921 -----+-----+-----+-----+-----+-----+ 1980  
GGAACCCAACCCCGTTACCACTGCTCGACACCCTTCTCGGGACCTATCCTTCGGTGACTC  
  
TCTGCCCTGGGCTCTGATAAAACCTTCACCATTAACTTGCTGTGTGACCTTGGGCATGTG  
1981 -----+-----+-----+-----+-----+-----+ 2040  
AGACGGGACCCGAGACTATTTTGGAAGTGGTAATTGAACGACACACTGGAACCCGTACAC  
  
GCTTTCCCTCTCTGGCCTCAGTCTGTTTCATCTCCCAAATGGATAATGAAGCCTCTTGGA  
2041 -----+-----+-----+-----+-----+-----+ 2100  
CGAAAGGGAGAGACCGGAGTCAGACAAGTAGAGGGTTTACCTATTACTTCGGAGAACCCT  
  
GGCCCTACCATAGGATCTGTTGCCATGCTCAAATGAGTTACTGAATAAGGTGCTTCTGCT  
2101 -----+-----+-----+-----+-----+-----+ 2160  
CCGGGATGGTATCCTAGACAACGGTACGAGTTTACTCAATGACTTATTCACGAAGACGA  
  
TCTTCTAGAGATGGTGCTAAAGAAAGGACTAGCATATGAGACTTCTGGTACCAATGGGGC  
2161 -----+-----+-----+-----+-----+-----+ 2220  
AGAAGATCTCTACCACGATTTCTTTCCTGATCGTATACTCTGAAGACCATGGTTACCCCG  
  
TGGTGGGCATGCTGTCCACTGTGTGGTGCTAGGACTGCCAATGCCAGGCCCAAGGGACAA  
2221 -----+-----+-----+-----+-----+-----+ 2280  
ACCACCCGTACGACAGGTGACACACCACGATCCTGACGGTTACGGTCCGGGTTCCTGTT  
  
AAAGAACAGAGCTTTTTGTTCTCATGGCTGGCCCTGCTACCTCCGAGGCACCCTGCAGGG  
2281 -----+-----+-----+-----+-----+-----+ 2340  
TTTCTTGTCTCGAAAAACAAGAGTACCGACCGGGACGATGGAGGCTCCGTGGGACGTCCC  
  
CAATGCATGTCATCCCAACCCCCACACTCCCCATCCTCCAACCCACTGGTCTCATGCCCA  
2341 -----+-----+-----+-----+-----+-----+ 2400  
GTTACGTACAGTAGGGTTGGGGGTGTGAGGGGTAGGAGGTTGGGTGACCAGAGTACGGGT

FIGURE 2

Sheet 4 of 5



AAGAAGAGTTGAAGGCATGGGAGCCAACATTTTATTGAAGAAGCCACAGAGGCTGAAATT  
2401 -----+-----+-----+-----+-----+-----+ 2460  
TTCTTCTCAACTTCCGTACCCTCGGTGTAAAATAACTTCTTCGGTGTCTCCGACTTTAA  
  
CAATAAACACAAGTTTTATGAGTAAAAAAAAAAAAAAAAAAAA  
2461 -----+-----+-----+-----+-----+ 2501  
GTTATTTGTGTTCAAATACTCATTTTTTTTTTTTTTTTTTTT

FIGURE 2

Sheet 5 of 5

CTGCACCTGAAGCATTGTTGGTGGGTGAGCAGCATGGGCTTTGAGGAGCTGCTGGAGCAGGT  
1 -----+-----+-----+-----+-----+-----+ 60  
GACGTGGACTTCGTAAACCACCCACTCGTCGTACCCGAAACTCCTCGACGACCTCGTCCA  
M G F E E L L E Q V -  
GGGCGGCTTTGGGCCCTTCCAAGTGGCAATGTGGCACTGCTGGCCCTGCCCCGAGTGCT  
61 -----+-----+-----+-----+-----+-----+ 120  
CCCGCCGAAACCCGGGAAGGTTGACGCCTTACACCGTGACGACCGGGACGGGGCTCACGA  
G G F G P F Q L R N V A L L A L P R V L -  
GCTACCACTGCACTTCCTCCTGCCCATCTTCTGGCTGCCGTGCCCTGCCACCGATGTGC  
121 -----+-----+-----+-----+-----+-----+ 180  
CGATGGTGACGTGAAGGAGGACGGGTAGAAGGACCGACGGCACGGACGGGTGGCTACACG  
L P L H F L L P I F L A A V P A H R C A -  
CCTGCCGGGTGCCCCCTGCCAAGTTCAGCCATCAGGATGTGTGGCTGGAGGCCCATCTTCC  
181 -----+-----+-----+-----+-----+-----+ 240  
GGACGGCCACGGGGACGGTTGAAGTCGGTAGTCCTACACACCGACCTCCGGGTAGAAGG  
L P G A P A N F S H Q D V W L E A H L P -  
CCGGGAGCCTGATGGCACGCTCAGCTCCTGCCTCCGCTTTGCCTATCCCCAGGCTCTCCC  
241 -----+-----+-----+-----+-----+-----+ 300  
GGCCCTCGGACTACCGTGCGAGTCGAGGACGGAGGCGAAACGGATAGGGGTCCGAGAGGG  
R E P D G T L S S C L R F A Y P Q A L P -  
CAACACCACGTTGGGGGAAGAAAGGCAGAGCCGTGGGGAGCTGGAGGATGAACCTGCCAC  
301 -----+-----+-----+-----+-----+-----+ 360  
GTTGTGGTGCAACCCCCTTCTTCCGTCTCGGCACCCCTCGACCTCCTACTTGGACGGTG  
N T T L G E E R Q S R G E L E D E P A T -  
AGTGCCCTGCTCTCAGGGCTGGGAGTACGACCACTCAGAATTCTCCTCTACCATTGCAAC  
361 -----+-----+-----+-----+-----+-----+ 420  
TCACGGGACGAGAGTCCCGACCCTCATGCTGGTGAGTCTTAAGAGGAGATGGTAACGTTG  
V P C S Q G W E Y D H S E F S S T I A T -  
TGAGTGGGATCTGGTGTGTGAGCAGAAAGGTCTGAACAGAGCTGCGTCCACTTTCTTCTT  
421 -----+-----+-----+-----+-----+-----+ 480  
ACTCACCCCTAGACCACACACTCGTCTTCCAGACTTGTCTCGACGCAGGTGAAAGAAGAA  
E W D L V C E Q K G L N R A A S T F F F -  
CGCCGGTGTGCTGGTGGGGGCTGTGGCCTTTGGATATCTGTCCGACAGGTTTGGGCGGCG  
481 -----+-----+-----+-----+-----+-----+ 540  
GCGGCCACACGACCACCCCGACACCGGAAACCTATAGACAGGCTGTCCAAACCCGCGC  
A G V L V G A V A F G Y L S D R F G R R -

FIGURE 3  
Sheet 1 of 4

541 GCGTCTGCTGCTGGTAGCCTACGTGAGTACCCTGGTGCTGGGCCTGGCATCTGCAGCCTC  
-----+-----+-----+-----+-----+ 600  
CGCAGACGACGACCATCGGATGCACTCATGGGACCACGACCCGGACCGTAGACGTCGGAG  
R L L L V A Y V S T L V L G L A S A A S -  
CGTCAGCTATGTAATGTTTGCCATCACCCGCACCCTTACTGGCTCAGCCCTGGCTGGTTT  
601 -----+-----+-----+-----+-----+ 660  
GCAGTCGATACATTACAAACGGTAGTGGGCGTGGAATGACCGAGTCGGGACCGACCAAA  
V S Y V M F A I T R T L T G S A L A G F -  
TACCATCATCGTGATGCCACTGGAGCTGGAGTGGCTGGATGTGGAGCACCGCACCGTGGC  
661 -----+-----+-----+-----+-----+ 720  
ATGGTAGTAGCACTACGGTGACCTCGACCTCACCGACCTACACCTCGTGGCGTGGCACCG  
T I I V M P L E L E W L D V E H R T V A -  
TGGAGTCCTGAGCAGCACCTTCTGGACAGGGGGCGTGATGCTGCTGGCACTGGTTGGGTA  
721 -----+-----+-----+-----+-----+ 780  
ACCTCAGGACTCGTCGTGGAAGACCTGTCCCCCGCACTACGACGACCGTGACCAACCCAT  
G V L S S T F W T G G V M L L A L V G Y -  
CCTGATACGGGACTGGCGATGGCTTCTGCTAGCTGTCACCCTGCCTTGTGCCCCAAGCAT  
781 -----+-----+-----+-----+-----+ 840  
GGACTATGCCCTGACCGCTACCGAAGACGATCGACAGTGGGACGGAACACGGGGTTCGTA  
L I R D W R W L L L A V T L P C A P S I -  
CCTCAGCCTCTGGTGGGTGCCTGAGTCTGCACGCTGGCTTCTGACCCAAGGCCATGTGAA  
841 -----+-----+-----+-----+-----+ 900  
GGAGTCGGAGACCACCCACGACTCAGACGTGCGACCGAAGACTGGGTTCGGGTACACTT  
L S L W W V P E S A R W L L T Q G H V K -  
AGAGGCCACAGGTACTTGCTCCACTGTGCCAGGCTCAATGGGCGGCCAGTGTGTGAGGA  
901 -----+-----+-----+-----+-----+ 960  
TCTCCGGGTGTCCATGAACGAGGTGACACGGTCCGAGTTACCCGCCGGTCACACACTCCT  
E A H R Y L L H C A R L N G R P V C E D -  
CAGCTTCAGCCAGGAGGCTGTGAGCAAAGTGGCCGCCGGGGAACGGGTGGTCCGAAGACC  
961 -----+-----+-----+-----+-----+ 1020  
GTCGAAGTCGGTCCTCCGACACTCGTTTCACCGGCGGCCCCCTTGCCCACCAGGCTTCTGG  
S F S Q E A V S K V A A G E R V V R R P -  
TTCATACCTAGACCTGTTCCGCACACCACGGCTCCGACACATCTCACTGTGCTGCGTGGT  
1021 -----+-----+-----+-----+-----+ 1080  
AAGTATGGATCTGGACAAGGCGTGTGGTGCCGAGGCTGTGTAGAGTGACACGACGCACCA  
S Y L D L F R T P R L R H I S L C C V V -

FIGURE 3  
Sheet 2 of 4

GGTGTGGTTCGGAGTGAACCTTCTCCTATTACGGCCTGAGTCTGGATGTGTCGGGGCTGGG  
1081 -----+-----+-----+-----+-----+-----+ 1140  
CCACACCAAGCCTCACTTGAAGAGGATAATGCCGGACTCAGACCTACACAGCCCCGACCC  
  
V W F G V N F S Y Y G L S L D V S G L G -  
  
GCTGAACGTGTACCAGACACAGCTGTTGTTTCGGGGCTGTGGAAGTGGCCTCCAAGCTGCT  
1141 -----+-----+-----+-----+-----+-----+ 1200  
CGACTTGACATGGTCTGTGTGCGACAACAAGCCCCGACACCTTGACGGGAGGTTCGACGA  
  
L N V Y Q T Q L L F G A V E L P S K L L -  
  
GGTCTACTTGTTCGGTGGCTACGCAGGACGCCGCCTCACGCAAGCCGGGACACTGCTGGG  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CCAGATGAACAGCCACGCGATGCGTCCTGCGGCGGAGTGCCTTCGGCCCTGTGACGACCC  
  
V Y L S V R Y A G R R L T Q A G T L L G -  
  
CACGGCCCTGGCGTTCGGCACTAGACTGCTAGTGTCTCCGATATGAAGTCCTGGAGCAC  
1261 -----+-----+-----+-----+-----+-----+ 1320  
GTGCCGGGACCGCAAGCCGTGATCTGACGATCACAGGAGGCTATACTTCAGGACCTCGTG  
  
T A L A F G T R L L V S S D M K S W S T -  
  
TGTCTGGCAGTGATGGGGAAAGCTTTTCTGAAGCTGCCTTCACCACTGCCTACCTGTT  
1321 -----+-----+-----+-----+-----+-----+ 1380  
ACAGGACCGTCACTACCCCTTTCGAAAAAGACTTCGACGGAAGTGGTGACGGATGGACAA  
  
V L A V M G K A F S E A A F T T A Y L F -  
  
CACTTCAGAGTTGTACCCTACGGTGCTCAGACAGACAGGGATGGGGCTGACTGCACTGGT  
1381 -----+-----+-----+-----+-----+-----+ 1440  
GTGAAGTCTCAACATGGGATGCCACGAGTCTGTCTGTCCCTACCCCGACTGACGTGACCA  
  
T S E L Y P T V L R Q T G M G L T A L V -  
  
GGGCCGGCTGGGGGGCTCTTTGGCCCCACTGGCGGCCTTGCTGGATGGAGTGTGGCTGTC  
1441 -----+-----+-----+-----+-----+-----+ 1500  
CCCGGCCGACCCCCCGAGAAACCGGGGTGACCGCCGGAACGACCTACCTCACACCGACAG  
  
G R L G G S L A P L A A L L D G V W L S -  
  
ACTGCCCAAGCTTACTTATGGGGGGATCGCCCTGCTGGCTGCCGGCACC GCCCTCCTGCT  
1501 -----+-----+-----+-----+-----+-----+ 1560  
TGACGGGTTCGAATGAATACCCCTAGCGGGACGACCGACGGCCGTGGCGGGAGGACGA  
  
L P K L T Y G G I A L L A A G T A L L L -  
  
GCCAGAGACGAGGCAGGCACAGCTGCCAGAGACCATCCAGGACGTGGAGAGAAAGAGAGA  
1561 -----+-----+-----+-----+-----+-----+ 1620  
CGGTCTCTGCTCCGTCCGTGTCGACGGTCTCTGGTAGGTCTGACCTCTCTTTCTCTCT  
  
P E T R Q A Q L P E T I Q D V E R K R D -

FIGURE 3  
Sheet 3 of 4

1621 TGGTGCTAAAGAAAGGACTAGCATATGAGACTTCTGGTACCAATGGGGCTGGTGGGCATG 1680  
-----+-----+-----+-----+-----+-----+  
ACCACGATTTCTTTCCTGATCGTATACTCTGAAGACCATGGTTACCCCGACCACCCGTAC  
  
G A K E R T S I \*  
  
1681 CTGTCCACTGTGTGGTGCTAGGACTGCCAATGCCAGGCCCAAGGGACAAAAAGAACAGAG 1740  
-----+-----+-----+-----+-----+-----+  
GACAGGTGACACACCACGATCCTGACGGTTACGGTCCGGGTTCCCTGTTTTTCTTGTC  
  
1741 CTTTTTGTTCATGGCTGGCCCTGCTACCTCCGAGGCACCCTGCAGGGCAATGCATGTC 1800  
-----+-----+-----+-----+-----+-----+  
GAAAAACAAGAGTACCGACCGGGACGATGGAGGCTCCGTGGGACGTCCCGTTACGTACAG  
  
1801 ATCCCAACCCCCACACTCCCCATCCTCCAACCCACTGGTCTCATGCCCAAAGAAGAGTTG 1860  
-----+-----+-----+-----+-----+-----+  
TAGGGTTGGGGGTGTGAGGGGTAGGAGGTTGGGTGACCAGAGTACGGGTTTCTTCTCAAC  
  
1861 AAGGCATGGGAGCCAACATTTTATTGAAGAAGCCACAGAGGCTGAAATTCAATAAACACA 1920  
-----+-----+-----+-----+-----+-----+  
TTCCGTACCCTCGGTTGTAAAATAACTTCTTCGGTGTCTCCGACTTTAAGTTATTTGTGT  
  
1921 AGTTTTATGAGTAAAAAAAAAAAAAAAAAAAAA 1950  
-----+-----+-----+  
TCAAAATACTCATTTTTTTTTTTTTTTTTTTT

FIGURE 3  
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GCAGGGACCTCAACTACACTGATCACCAGCCCCATCGGATCCAGACCCGGCCACCAGTGC  
1 -----+-----+-----+-----+-----+-----+ 60  
CGTCCCTGGAGTTGATGTGACTAGTGGTCGGGGTAGCCTAGGTCTGGGCCGGTGGTCACG  
  
CATGACCTTCTCGGAGATCCTGGACCGTGTGGGAAGCATGGGCCATTTCCAGTTCCTGCA  
61 -----+-----+-----+-----+-----+-----+ 120  
GTACTGGAAGAGCCTCTAGGACCTGGCACACCCTTCGTACCCGGTAAAGGTCAAGGACGT  
  
M T F S E I L D R V G S M G H F Q F L H -  
  
TG TAGCCATACTGGGCCTCCCGATCCTCAACATGGCCAACCACAACCTGCTGCAGATCTT  
121 -----+-----+-----+-----+-----+-----+ 180  
ACATCGGTATGACCCGGAGGGCTAGGAGTTGTACCGGTTGGTGTGGACGACGTCTAGAA  
  
V A I L G L P I L N M A N H N L L Q I F -  
  
CACAGCCGCCACCCCTGTCCACCACTGTCGCCCCGCCCCACAATGCCTCCACAGGGCCTTG  
181 -----+-----+-----+-----+-----+-----+ 240  
GTGTCGGCGGTGGGGACAGGTGGTGACAGCGGGCGGGGTGTTACGGAGGTGTCCCGGAAC  
  
T A A T P V H H C R P P H N A S T G P W -  
  
GGTGCTCCCCATGGGCCCAAATGGGAAGCCTGAGAGGTGCCTCCGTTTGTACATCCGCC  
241 -----+-----+-----+-----+-----+-----+ 300  
CCACGAGGGGTACCCGGGTTTACCCTTCGGACTCTCCACGGAGGCAAAACATGTAGGCGG  
  
V L P M G P N G K P E R C L R F V H P P -  
  
CAATGCCAGCCTGCCCAATGACACCCAGAGGGCCATGGAGCCATGCCTGGATGGCTGGGT  
301 -----+-----+-----+-----+-----+-----+ 360  
GTTACGGTCGGACGGGTTACTGTGGGTCTCCCGGTACCTCGGTACGGACCTACCGACCCA  
  
N A S L P N D T Q R A M E P C L D G W V -  
  
CTACAACAGCACCAAGGACTCCATTGTGACAGAGTGGGACTTGGTGTGCAACTCCAACAA  
361 -----+-----+-----+-----+-----+-----+ 420  
GATGTTGTCGTGGTTCCTGAGGTAACACTGTCTCACCCTGAACCACACGTTGAGGTTGTT  
  
Y N S T K D S I V T E W D L V C N S N K -  
  
ACTGAAGGAGATGGCCCAGTCTATCTTCATGGCAGGTATACTGATTGGAGGGCTCGTGCT  
421 -----+-----+-----+-----+-----+-----+ 480  
TGACTTCCTCTACCGGGTCAGATAGAAGTACCGTCCATATGACTAACCTCCCGAGCACGA  
  
L K E M A Q S I F M A G I L I G G L V L -  
  
TGGAGACCTGTCTGACAGGTTTGGCCGCAGGCCCATCCTGACCTGCAGCTACCTGCTGCT  
481 -----+-----+-----+-----+-----+-----+ 540  
ACCTCTGGACAGACTGTCCAAACCGGCGTCCGGGTAGGACTGGACGTCGATGGACGACGA  
  
G D L S D R F G R R P I L T C S Y L L L -  
  
GGCAGCCAGCGGCTCCGGTGCAGCCTTCAGCCCCACCTTCCCCATCTACATGGTCTTCCG  
541 -----+-----+-----+-----+-----+-----+ 600

FIGURE 4  
Sheet 1 of 4

CCGTCGGTCGCCGAGGCCACGTCGGAAGTCGGGGTGGGAAGGGGTAGATGTACCAGAAGGC  
A A S G S G A A F S P T F P I Y M V F R -  
CTTCCTGTGTGGCTTTGGCATCTCAGGCATTACCCTGAGCACCGTCATCTTGAATGTGGA  
601 -----+-----+-----+-----+-----+-----+ 660  
GAAGGACACACCGAAACCGTAGAGTCCGTAATGGGACTCGTGGCAGTAGAACTTACACCT  
F L C G F G I S G I T L S T V I L N V E -  
ATGGGTGCCTACCCGGATGCGGGCCATCATGTCGACAGCACTCGGGTACTGCTACACCTT  
661 -----+-----+-----+-----+-----+-----+ 720  
TACCCACGGATGGGCCTACGCCCCGGTAGTACAGCTGTCGTGAGCCCATGACGATGTGGAA  
W V P T R M R A I M S T A L G Y C Y T F -  
TGGCCAGTTCATTCTGCCCCGGCCTGGCCTACGCCATCCCCCAGTGGCGTTGGCTGCAGTT  
721 -----+-----+-----+-----+-----+-----+ 780  
ACCGGTCAAGTAAGACGGGCCGGACCGGATGCGGTAGGGGGTCAACGCAACCGACGTCAA  
G Q F I L P G L A Y A I P Q W R W L Q L -  
AACTGTGTCCATTCCCTTCTTCGTCTTCTTCCTATCATCCTGGTGGACACCAGAGTCCAT  
781 -----+-----+-----+-----+-----+-----+ 840  
TTGACACAGGTAAGGGAAGAAGCAGAAGAAGGATAGTAGGACCACCTGTGGTCTCAGGTA  
T V S I P F F V F F L S S W W T P E S I -  
ACGCTGGTTGGTCTTGTCTGGAAAGTCCTCGAAGGCCCTGAAGATACTCCGGCGGGTGGC  
841 -----+-----+-----+-----+-----+-----+ 900  
TGCGACCAACCAGAACAGACCTTTCAGGAGCTTCCGGGACTTCTATGAGGCCGCCACCG  
R W L V L S G K S S K A L K I L R R V A -  
TGTCTTCAATGGCAAGAAGGAAGAGGGAGAAAGGCTCAGCTTGGAGGAGCTCAAACCTCAA  
901 -----+-----+-----+-----+-----+-----+ 960  
ACAGAAGTTACCGTTCTTCTTCTCCCTCTTCCGAGTCGAACCTCCTCGAGTTTGAGTT  
V F N G K K E E G E R L S L E E L K L N -  
CCTGCAGAAGGAGATCTCCTTGGCCAAGGCCAAGTACACCGCAAGTGACCTGTTCCGGAT  
961 -----+-----+-----+-----+-----+-----+ 1020  
GGACGTCTTCTCTAGAGGAACCGGTTCCGGTTCATGTGGCGTTCACTGGACAAGGCCTA  
L Q K E I S L A K A K Y T A S D L F R I -  
ACCCATGCTGCGCCGCATGACCTTCTGTCTTCCCTGGCCTGGTTTGCTACCGGTTTTGC  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGGGTACGACGCGCGTACTGGAAGACAGAAAGGGACCGGACCAAACGATGGCCAAAACG  
P M L R R M T F C L S L A W F A T G F A -  
CTACTATAGTTTGGCTATGGGTGTGGAAGAATTTGGAGTCAACCTCTACATCCTCCAGAT  
1081 -----+-----+-----+-----+-----+-----+ 1140

FIGURE 4  
Sheet 2 of 4

GATGATATCAAACCGATACCCACACCTTCTTAAACCTCAGTTGGAGATGTAGGAGGTCTA  
Y Y S L A M G V E E F G V N L Y I L Q I -  
CATCTTTGGTGGGGTCGATGTCCCAGCCAAGTTCATCACCATCCTCTCCTTAAGCTACCT  
1141 -----+-----+-----+-----+-----+-----+-----+ 1200  
GTAGAAACCACCCCAGCTACAGGGTCGGTTCAAGTAGTGGTAGGAGAGGAATTTCGATGGA  
I F G G V D V P A K F I T I L S L S Y L -  
GGGCCGGCATACCACTCAGGCCGCTGCCCTGCTCCTGGCAGGAGGGGCCATCTTGGCTCT  
1201 -----+-----+-----+-----+-----+-----+-----+ 1260  
CCCGGCCGTATGGTGAGTCCGGCGACGGGACGAGGACCGTCCTCCCCGGTAGAACCGAGA  
G R H T T Q A A A L L L A G G A I L A L -  
CACCTTTGTGCCCTTGGACTTGCAGACCGTGAGGACAGTATTGGCTGTGTTTGGGAAGGG  
1261 -----+-----+-----+-----+-----+-----+-----+ 1320  
GTGGAAACACGGGAACCTGAACGTCTGGCACTCCTGTCATAACCGACACAAACCCTTCCC  
T F V P L D L Q T V R T V L A V F G K G -  
ATGCCTATCCAGCTCCTTCAGCTGCCTCTTCCTCTACACAAGTGAATTATACCCACAGT  
1321 -----+-----+-----+-----+-----+-----+-----+ 1380  
TACGGATAGGTTCGAGGAAGTCGACGGAGAAGGAGATGTGTTCACTTAATATGGGGTGTCA  
C L S S S F S C L F L Y T S E L Y P T V -  
CATCAGGCAAACAGGTATGGGCGTAAGTAACCTGTGGACCCGCGTGGGAAGCATGGTGTG  
1381 -----+-----+-----+-----+-----+-----+-----+ 1440  
GTAGTCCGTTTGTCCATACCCGCATTCATTGGACACCTGGGCGCACCTTCGTACCACAG  
I R Q T G M G V S N L W T R V G S M V S -  
CCCGCTGGTGAAAATCACGGGTGAGGTACAGCCCTTCATCCCCAATATCATCTACGGGAT  
1441 -----+-----+-----+-----+-----+-----+-----+ 1500  
GGGCGACCACTTTTAGTGCCCACTCCATGTCGGGAAGTAGGGGTTATAGTAGATGCCCTA  
P L V K I T G E V Q P F I P N I I Y G I -  
CACCGCCCTCCTCGGGGGCAGTGCTGCCCTCTTCCTGCCTGAGACCCTGAATCAGCCCTT  
1501 -----+-----+-----+-----+-----+-----+-----+ 1560  
GTGGCGGGAGGAGCCCCCGTCACGACGGGAGAAGGACGGAAGTCTGGGACTTAGTCGGGAA  
T A L L G G S A A L F L P E T L N Q P L -  
GCCAGAGACTATCGAAGACCTGGAAAAGTGGTCCCTGCGGGCAAAGAAGCCAAAGCAGGA  
1561 -----+-----+-----+-----+-----+-----+-----+ 1620  
CGGTCTCTGATAGCTTCTGGACCTTTTGACCAGGGACGCCCGTTTCTTCGGTTTCGTCCT  
P E T I E D L E N W S L R A K K P K Q E -  
GCCAGAGGTGGAAAAGGCCTCCAGAGGATCCCTCTACAGCCTCACGGACCAGGCCTGGG  
1621 -----+-----+-----+-----+-----+-----+-----+ 1680

FIGURE 4  
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CGGTCTCCACCTTTTCCGGAGGGTCTCCTAGGGAGATGTCGGAGTGCCTGGTCCGGACCC  
P E V E K A S Q R I P L Q P H G P G L G -  
CTCCAGCTGAGGACAACGGAACCCCCTTTCCCTGCCCTCCAGAGACTGATCCTAGCCAGG  
1581 -----+-----+-----+-----+-----+-----+ 1740  
GAGGTGCGACTCCTGTTGCCTTGGGGGAAAGGGACGGGAGGTCTCTGACTAGGATCGGTCC  
S S \*  
CACCTTAGGAGTATAGGGAGGCCCCATATAGGTCCATCCTCCTAGGATGAAGCCTTCTGA  
1741 -----+-----+-----+-----+-----+-----+ 1800  
GTGGAATCCTCATATCCCTCCGGGGTATATCCAGGTAGGAGGATCCTACTTCGGAAGACT  
GAGCTTGGTGAAGGTGTCTCCATCACCACCACCAGAGCCTCCTGCCCAGCCCTGGCCAGT  
1801 -----+-----+-----+-----+-----+-----+ 1860  
CTCGAACCCTTCCACAGAGGTAGTGGTGGTGGTCTCGGAGGACGGGTCCGGACCGGTCA  
TCAAAGGTTCAAGCCATCCCTGCCCTTGTCTCCCTGCAACCCAAGCCCTGCCATTCTTC  
1861 -----+-----+-----+-----+-----+-----+ 1920  
AGTTTCCAAGTTCGGTAGGGACGGGAACAAGAGGGACGTTGGGTTCGGGACGGTAAGAAG  
TGTCTAGCCCTTCCCCACTGGCCAACCTCCCCCACTGTCCCGGTCTCTTCCCCTGAGGT  
1921 -----+-----+-----+-----+-----+-----+ 1980  
ACAGATCGGGAAGGGGTGACCGGTTGAAGGGGGTGACAGGGCCAGGAGAAGGGGACTCCA  
CCCCTGATATCCCCTGGCTCAGTCCTAACAAGACTGAGTCTTAACAAGATGAGAAGTCCT  
1981 -----+-----+-----+-----+-----+-----+ 2040  
GGGGACTATAGGGGACCGAGTCAGGATTGTTCTGACTCAGAATTGTTCTACTCTTCAGGA  
CCCCTTCTTGCCTCCCACACTTTTCTTTGATGGGAGGTTTCAATAAACAGCGATAAGAAC  
2041 -----+-----+-----+-----+-----+-----+ 2100  
GGGGAAGAACGGAGGGGTGTGAAAAGAACTACCCTCCAAAGTTATTTGTCGCTATTCTTG  
TCTAAAAAAAAAAAAAAAAAAAAA  
2101 -----+-----+ 2121  
AGATTTTTTTTTTTTTTTTTTT

FIGURE 4  
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CAAATTATTTCTTACGTGACTTTAGAGAAAACGGCTACCTATCTGACCCCAAACGACTT  
1 -----+-----+-----+-----+-----+-----+ 60  
GTTTAATAAAGAATGCACTGAAATCTCTTTTGCCGATGGATAGACTGGGGTTTTGCTGAA  
GAGGAAACTGTTTCCACGGTCCTGCTGCAGGGGGGAAGCACAGTCGTCAAGAAGAGAGTG  
61 -----+-----+-----+-----+-----+-----+ 120  
CTCCTTTGACAAAGGTGCCAGGACGACGTCCCCCTTCGTGTCAGCAGTTCTTCTCTCAC  
GGGTCAGGATCAAAACACATTTAGTGTGACTTAGGGAAAGAAAACATTTTCCCTCTTTGA  
121 -----+-----+-----+-----+-----+-----+ 180  
CCCAGTCCTAGTTTTGTGTAAATCACACTGAATCCCTTTCTTTTGTAAGGGAGAACT  
ACCTCTCTGGATACAGTCATTTTGCCTCTACTTGAGGATCAACTGTTCAACCTCAATGGC  
181 -----+-----+-----+-----+-----+-----+ 240  
TGGAGAGACCTATGTCAGTAAAACGGAGATGAACTCCTAGTTGACAAGTTGGAGTTACCG  
M A -  
CTTTCAGGACCTCCTGGGTCACGCTGGTGACCTGTGGAGATTCCAGATCCTTCAGACTGT  
241 -----+-----+-----+-----+-----+-----+ 300  
GAAAGTCCTGGAGGACCCAGTGCGACCACTGGACACCTCTAAGGTCTAGGAAGTCTGACA  
F Q D L L G H A G D L W R F Q I L Q T V -  
TTTTCTCTCAATCTTTGCTGTTGCTACATACCTTCATTTTATGCTGGAGAACTTCACTGC  
301 -----+-----+-----+-----+-----+-----+ 360  
AAAAGAGAGTTAGAAACGACAACGATGTATGGAAGTAAAATACGACCTCTTGAAGTGACG  
F L S I F A V A T Y L H F M L E N F T A -  
ATTCATACCTGGCCATCGCTGCTGGGTCCACATCCTGGACAATGACACTGTCTCTGACAA  
361 -----+-----+-----+-----+-----+-----+ 420  
TAAGTATGGACCGGTAGCGACGACCCAGGTGTAGGACCTGTTACTGTGACAGAGACTGTT  
F I P G H R C W V H I L D N D T V S D N -  
TGACACTGGGGCCCTCAGCCAAGATGCACTCTTGAGAATCTCCATCCCCTGGACTCAAA  
421 -----+-----+-----+-----+-----+-----+ 480  
ACTGTGACCCCGGGAGTCGGTTCTACGTGAGAACTCTTAGAGGTAGGGTGACCTGAGTTT  
D T G A L S Q D A L L R I S I P L D S N -  
CATGAGGCCAGAGAAGTGTCGTCGCTTTGTTTCATCCTCAGTGGCAGCTCCTTCACCTGAA  
481 -----+-----+-----+-----+-----+-----+ 540  
GTACTCCGGTCTCTTCACAGCAGCGAAACAAGTAGGAGTCACCGTCGAGGAAGTGGACTT  
M R P E K C R R F V H P Q W Q L L H L N -  
TGGGACCTTCCCAACACAAGTGACGCAGACATGGAGCCCTGTGTGGATGGCTGGGTGTA  
541 -----+-----+-----+-----+-----+-----+ 600  
ACCCTGGAAGGGGTGTGTTCACTGCGTCTGTACCTCGGGACACACCTACCGACCCACAT  
G T F P N T S D A D M E P C V D G W V Y -

FIGURE 5  
Sheet 1 of 4



501 TGACAGAATCTCCTTCTCATCCACCATCGGTGACCTGAAGTGGGATCTGGTATGTGACTC  
-----+-----+-----+-----+-----+-----+ 660  
ACTGTCTTAGAGGAAGAGTAGGTGGTAGCCACTGGACTTCACCCTAGACCATACTGAG  
  
D R I S F S S T I G D L K W D L V C D S -  
  
561 TCAATCACTGACTTCAGTGGCTAAATTTGTATTTCATGGCTGGAATGATGTTGGGAGGCAT  
-----+-----+-----+-----+-----+-----+ 720  
AGTTAGTGACTGAAGTCACCGATTAAACATAAGTACCGACCTTACTACAACCCTCCGTA  
  
Q S L T S V A K F V F M A G M M L G G I -  
  
721 CTTAGGCGTTCATTTATCAGACAGGTTTGGGAGAAGTTTCGTGCTCAGATGGTGTACCT  
-----+-----+-----+-----+-----+-----+ 780  
GAATCCGCAAGTAAATAGTCTGTCCAAACCCTCTTCAAAGCACGAGTCTACCACAATGGA  
  
L G V H L S D R F G R S F V L R W C Y L -  
  
781 CCAGGTTGCCATTGTTGGCACTTGTGCAGCGTTGGCTCCCACTTTCCTCATTTACTGCTC  
-----+-----+-----+-----+-----+-----+ 840  
GGTCCAACGGTAACAACCGTGAACACGTCGCAACCGAGGGTGAAAGGAGTAAATGACGAG  
  
Q V A I V G T C A A L A P T F L I Y C S -  
  
841 AGTACGCTTCTTGTCTGGGATTGCTGCAATGAGCTTCATAACAAATACTATTATGTTAAT  
-----+-----+-----+-----+-----+-----+ 900  
TCATGCGAAGAACAGACCCTAACGACGTTACTCGAAGTATTGTTTATGATAATAACAATTA  
  
V R F L S G I A A M S F I T N T I M L I -  
  
901 AGCCGAGTGGGCAACACACAGATTCCAGGCCATGGGAATTACATTGGGAATGTGCCCTTC  
-----+-----+-----+-----+-----+-----+ 960  
TCGGCTCACCCGTTGTGTGTCTAAGGTCCGGTACCCTTAATGTAACCCTTACACGGGAAG  
  
A E W A T H R F Q A M G I T L G M C P S -  
  
961 TGGTATTGCATTTATGACCCTGGCAGGCCTGGCTTTTGCCATTCGAGACTGGCATATCCT  
-----+-----+-----+-----+-----+-----+ 1020  
ACCATAACGTAAATACTGGGACCGTCCGGACCGAAAACGGTAAGCTCTGACCGTATAGGA  
  
G I A F M T L A G L A F A I R D W H I L -  
  
1021 CCAGCTGGTGGTGTCTGTACCATACTTTGTGATCTTTCTGACCTCAAGTTGGCTGCTAGA  
-----+-----+-----+-----+-----+-----+ 1080  
GGTCGACCACCACAGACATGGTATGAAACACTAGAAAGACTGGAGTTCAACCGACGATCT  
  
Q L V V S V P Y F V I F L T S S W L L E -  
  
1081 GTCTGCTCGGTGGCTCATTATCAACAATAAACCAGAGGAAGGCTTAAAGGAACTTAGAAA  
-----+-----+-----+-----+-----+-----+ 1140  
CAGACGAGCCACCGAGTAATAGTTGTTATTTGGTCTCCTTCCGAATTCCTTGAATCTTT  
  
S A R W L I I N N K P E E G L K E L R K -

FIGURE 5  
Sheet 2 of 4

AGCTGCACACAGGAGTGAATGAAGAATGCCAGAGACACCCTAACCTGGAGATTTTGAA  
1141 -----+-----+-----+-----+-----+-----+ 1200  
TCGACGTGTGTCCTCACCTTACTTCTTACGGTCTCTGTGGGATTGGGACCTCTAAACTT  
  
A A H R S G M K N A R D T L T L E I L K -  
  
ATCCACCATGAAAAAGAACTGGAGGCAGCACAAAAAAAACCTTCTCTGTGTGAAAT  
1201 -----+-----+-----+-----+-----+-----+ 1260  
TAGGTGGTACTTTTTTCTTGACCTCCGTCGTGTTTTTTTTTTTGAAGAGACACTTTA  
  
S T M K K E L E A A Q K K K P S L C E M -  
  
GCTCCACATGCCCAACATATGTAAAAGGATCTCCCTCCTGTCCTTTACGAGATTTGCAA  
1261 -----+-----+-----+-----+-----+-----+ 1320  
CGAGGTGTACGGGTTGTATACATTTTCTAGAGGGAGGACAGGAAATGCTCTAAACGTTT  
  
L H M P N I C K R I S L L S F T R F A N -  
  
CTTTATGGCCTATTTTGGCCTTAATCTCCATGTCCAGCATCTGGGGAACAATGTTTTCT  
1321 -----+-----+-----+-----+-----+-----+ 1380  
GAAATACCGGATAAAACCGGAATTAGAGGTACAGGTCGTAGACCCCTTGTTACAAAAGGA  
  
F M A Y F G L N L H V Q H L G N N V F L -  
  
GTTGCAGACTCTCTTTGGTGCAGTCATCCTCCTGGCCAACTGTGTTGCACCTTGGGCACT  
1381 -----+-----+-----+-----+-----+-----+ 1440  
CAACGTCTGAGAGAAACCACGTCAGTAGGAGGACCGGTTGACACAACGTGGAACCCGTGA  
  
L Q T L F G A V I L L A N C V A P W A L -  
  
GAAATACATGAACCGTCGAGCAAGCCAGATGCTTCTCATGTTTCTACTGGCAATCTGCCT  
1441 -----+-----+-----+-----+-----+-----+ 1500  
CTTTATGTACTTGGCAGCTCGTTTCGGTCTACGAAGAGTACAAGGATGACCGTTAGACGGA  
  
K Y M N R R A S Q M L L M F L L A I C L -  
  
TCTGGCCATCATATTTGTGCCACAAGAAATGCAGACGCTGCGTGAGGTTTTGGCAACACT  
1501 -----+-----+-----+-----+-----+-----+ 1560  
AGACCGGTAGTATAAACACGGTGTCTTTACGTCTGCGACGCACTCCAAAACCGTTGTGA  
  
L A I I F V P Q E M Q T L R E V L A T L -  
  
GGGCTTAGGAGCGTCTGCTCTTGCCAATACCCTTGCTTTTGCCCATGGAAATGAAGTAAT  
1561 -----+-----+-----+-----+-----+-----+ 1620  
CCCGAATCCTCGCAGACGAGAACGGTTATGGGAACGAAAACGGGTACCTTTACTTCATTA  
  
G L G A S A L A N T L A F A H G N E V I -  
  
TCCCACCATAATCAGGGCAAGAGCTATGGGGATCAATGCAACCTTTGCTAATATAGCAGG  
1621 -----+-----+-----+-----+-----+-----+ 1680  
AGGGTGGTATTAGTCCCGTTCTCGATACCCCTAGTTACGTTGGAACGATTATATCGTCC  
  
P T I I R A R A M G I N A T F A N I A G -

FIGURE 5  
Sheet 3 of 4

AGCCCTGGCTCCCCTCATGATGATCCTAAGTGTGTATTCTCCACCCCTGCCCTGGATCAT  
1681 -----+-----+-----+-----+-----+-----+ 1740  
TCGGGACCGAGGGGAGTACTACTAGGATTACACATAAGAGGTGGGGACGGGACCTAGTA  
  
A L A P L M M I L S V Y S P P L P W I I -  
  
CTATGGAGTCTTCCCCTTCATCTCTGGCTTTGCTTTCCTCCTCCTTCCTGAAACCAGGAA  
1741 -----+-----+-----+-----+-----+-----+ 1800  
GATACCTCAGAAGGGGAAGTAGAGACCGAAACGAAAGGAGGAGGAAGGACTTTGGTCCTT  
  
Y G V F P F I S G F A F L L L P E T R N -  
  
CAAGCCTCTGTTTGACACCATCCAGGATGAGAAAAATGAGAGAAAAGACCCCAGAGAACC  
1801 -----+-----+-----+-----+-----+-----+ 1860  
GTTTCGGAGACAAACTGTGGTAGGTCCTACTCTTTTACTCTCTTTTCTGGGGTCTCTTGG  
  
K P L F D T I Q D E K N E R K D P R E P -  
  
AAAGCAAGAGGATCCGAGAGTGGAAGTGACGCAGTTTTAAGGAATTCCAGGAGCTGACTG  
1861 -----+-----+-----+-----+-----+-----+ 1920  
TTTCGTTCTCCTAGGCTCTCACCTTCACTGCGTCAAAATTCCTTAAGGTCCTCGACTGAC  
  
K Q E D P R V E V T Q F \*  
  
CCGATCAATGAGCCAGATGAAGGGAACAATCAGGACTATTCCTAGACACTAGCAAAA  
1921 -----+-----+-----+-----+-----+-----+ 1977  
GGCTAGTTACTCGGTCTACTTCCCTTGTTAGTCCTGATAAGGATCTGTGATCGTTTT

FIGURE 5  
Sheet 4 of 4

CTCCTGATAGCAAAAGAACTGAGGAAGCTCTTTCCACTACGGCTGTATTGCACTGGTGAG  
1 -----+-----+-----+-----+-----+-----+ 60  
GAGGACTATCGTTTTCTTGACTCCTTCGAGAAAGGTGATGCCGACATAACGTGACCACTC  
TCCGGGCCCCATGGATGAGAAATTGATGCGAGGATCAATACAAGCTTAATTTGAATTAATA  
61 -----+-----+-----+-----+-----+-----+ 120  
AGGCCCGGGTACCTACTCTTTAACTACGCTCCTAGTTATGTTCTGAATTAACTTAATTAT  
AAAGGAAATATTTTCTCCCTTTGAACTTATCTCCGTAAAGCCATTGTGCCTCCTCTTGGG  
121 -----+-----+-----+-----+-----+-----+ 180  
TTTCCTTTATAAAAGAGGGAACTTGAATAGAGGCATTTCTGGTAACACGGAGGAGAACCC  
GGTCACGTGTTTACAATCAATGGCCTTTGAGGAGCTCTTGAGTCAAGTTGGAGGCCTTGG  
181 -----+-----+-----+-----+-----+-----+ 240  
CCAGTGCACAAGTGTTAGTTACCGGAACTCCTCGAGAACTCAGTTCAACCTCCGGAACC  
M A F E E L L S Q V G G L G -  
GAGATTCAGATGCTTCATCTGGTTTTTATTCTTCCCTCTCTCATGTTATTAATCCCTCA  
241 -----+-----+-----+-----+-----+-----+ 300  
CTCTAAAGTCTACGAAGTAGACCAAAAATAAGAAGGGAGAGAGTACAATAATTAGGGAGT  
R F Q M L H L V F I L P S L M L L I P H -  
TATACTGCTAGAGAACTTTGCTGCAGCCATTCCTGGTCATCGTTGCTGGGTCCACATGCT  
301 -----+-----+-----+-----+-----+-----+ 360  
ATATGACGATCTCTTGAAACGACGTCGGTAAGGACCAGTAGCAACGACCCAGGTGTACGA  
I L L E N F A A A I P G H R C W V H M L -  
GGACAATAATACTGGATCTGGTAATGAACTGGAATCCTCAGTGAAGATGCCCTCTTGAG  
361 -----+-----+-----+-----+-----+-----+ 420  
CCTGTTATTATGACCTAGACCATTACTTTGACCTTAGGAGTCACTTCTACGGGAGAACTC  
D N N T G S G N E T G I L S E D A L L R -  
AATCTCTATCCCACTAGACTCAAATCTGAGGCCAGAGAAGTGTCGTCGCTTTGTCCATCC  
421 -----+-----+-----+-----+-----+-----+ 480  
TTAGAGATAGGGTGATCTGAGTTTAGACTCCGGTCTCTTCACAGCAGCGAAACAGGTAGG  
I S I P L D S N L R P E K C R R F V H P -  
CCAGTGGCAGCTTCTTCACCTGAATGGGACTATCCACAGCACAAGTGAGGCAGACACAGA  
481 -----+-----+-----+-----+-----+-----+ 540  
GGTCACCGTCGAAGAAGTGGACTTACCCTGATAGGTGTCGTGTTCACTCCGTCTGTGTCT  
Q W Q L L H L N G T I H S T S E A D T E -  
ACCCTGTGTGGATGGCTGGGTATATGATCAAAGCTACTTCCCTTCGACCATTGTGACTAA  
541 -----+-----+-----+-----+-----+-----+ 600  
TGGGACACACCTACCGACCCATATACTAGTTTCGATGAAGGGAAGCTGGTAACACTGATT  
P C V D G W V Y D Q S Y F P S T I V T K -

FIGURE 6  
Sheet 1 of 5

GTGGGACCTGGTATGTGATTATCAGTCACTGAAATCAGTGGTTCAATTCCTACTTCTGAC  
501 -----+-----+-----+-----+-----+-----+ 660  
CACCTGGACCATACTAATAAGTCAGTGACTTTAGTCACCAAGTTAAGGATGAAGACTG  
W D L V C D Y Q S L K S V V Q F L L L T -  
TGGAATGCTGGTGGGAGGCATCATAGGTGGCCATGTCTCAGACAGGTTTGGGCGAAGATT  
561 -----+-----+-----+-----+-----+-----+ 720  
ACCTTACGACCACCCTCCGTAGTATCCACCGGTACAGAGTCTGTCCAAACCCGCTTCTAA  
G M L V G G I I G G H V S D R F G R R F -  
TATTCTCAGATGGTGTGTTGCTCCAGCTTGCCATTACTGACACCTGCGCTGCCTTCGCTCC  
721 -----+-----+-----+-----+-----+-----+ 780  
ATAAGAGTCTACCACAAACGAGGTGCAACGGTAATGACTGTGGACGCGACGGAAGCGAGG  
I L R W C L L Q L A I T D T C A A F A P -  
CACCTTCCCTGTTTACTGTGTACTACGCTTCTTGGCAGGTTTTCTTCCATGATCATTAT  
781 -----+-----+-----+-----+-----+-----+ 840  
GTGGAAGGGACAAATGACACATGATGCGAAGAACCGTCCAAAAGAAGGTACTAGTAATA  
T F P V Y C V L R F L A G F S S M I I I -  
ATCAAATAATTCTTTGCCCATTAAGTGGATAAGGCCCAACTCTAAAGCCCTGGTAGT  
841 -----+-----+-----+-----+-----+-----+ 900  
TAGTTTATTAAGAAACGGGTAATGACTCACCTATTCCGGGTGAGATTTCCGGGACCATCA  
S N N S L P I T E W I R P N S K A L V V -  
AATATTGTCATCTGGTGGCCCTTAGTATTGGACAGATAATCCTGGGAGGCTTGGCTTATGT  
901 -----+-----+-----+-----+-----+-----+ 960  
TTATAACAGTAGACCACGGGAATCATAACCTGTCTATTAGGACCCTCCGAACCGAATACA  
I L S S G A L S I G Q I I L G G L A Y V -  
CTTCCGAGACTGGCAAACCCTGCACGTGGTGGCGTCAGTACCTTTCCTTGGCCTCCTTCT  
961 -----+-----+-----+-----+-----+-----+ 1020  
GAAGGCTCTGACCGTTTGGGACGTGCACCACCGCAGTCATGGAAAGGAACCGGAGGAAGA  
F R D W Q T L H V V A S V P F L G L L L -  
CCTTCAAAGGTGGCTGGTGAATCTGCTCGGTGGTTGATAATCACCAATAAACTAGATGA  
1021 -----+-----+-----+-----+-----+-----+ 1080  
GGAAGTTTCCACCGACCACCTTAGACGAGCCACCAACTATTAGTGGTTATTTGATCTACT  
L Q R W L V E S A R W L I I T N K L D E -  
GGGCTTAAAGGCACTTAGAAAAGTTGCACGCACAAATGGAATAAAGAATGCTGAAGAAAC  
1081 -----+-----+-----+-----+-----+-----+ 1140  
CCCGAATTTCCGTGAATCTTTTCAACGTGCGTGTTTACCTTATTTCTTACGACTTCTTTG  
G L K A L R K V A R T N G I K N A E E T -

FIGURE 6  
Sheet 2 of 5



CCTGAACATAGAGGTTGTAAGATCCACCATGCAGGAGGAGCTGGATGCAGCACAGACCAA  
1141 -----+-----+-----+-----+-----+-----+-----+ 1200  
GGACTTGTATCTCCAACATTCTAGGTGGTACGTCCTCCTCGACCTACGTCGTGTCTGGTT  
L N I E V V R S T M Q E E L D A A Q T K -  
AACTACTGTGTGTGACTTGTTCGCAACCCCAGTATGCGTAAAAGGATCTGTATCCTGGT  
1201 -----+-----+-----+-----+-----+-----+-----+ 1260  
TTGATGACACACACTGAACAAGGCGTTGGGGTCATACGCATTTTCCTAGACATAGGACCA  
T T V C D L F R N P S M R K R I C I L V -  
ATTTTGTGAGATTTGCAAACACAATACCTTTTTATGGTACCATGGTCAATCTTCAGCATGT  
1261 -----+-----+-----+-----+-----+-----+-----+ 1320  
TAAAACTCTAAACGTTTGTGTTATGGAAAAATACCATGGTACCAGTTAGAAGTCGTACA  
F L R F A N T I P F Y G T M V N L Q H V -  
GGGGAGCAACATTTTCCTGTTGCAGGTACTTTATGGAGCTGTCGCTCTCATAGTTCGATG  
1321 -----+-----+-----+-----+-----+-----+-----+ 1380  
CCCCTCGTTGTAAAAGGACAACGTCCATGAAATACCTCGACAGCGAGAGTATCAAGCTAC  
G S N I F L L Q V L Y G A V A L I V R C -  
TCTTGCTCTTTTGACACTAAATCATATGGGCCGTCGAATAAGCCAGATATTGTTTCATGTT  
1381 -----+-----+-----+-----+-----+-----+-----+ 1440  
AGAACGAGAAAACGTGTGATTTAGTATACCCGGCAGCTTATTCGGTCTATAACAAGTACAA  
L A L L T L N H M G R R I S Q I L F M F -  
CCTGGTGGGCCTTTCCATTTTGGCCAACACGTTTGTGCCCAAAGAAATGCAGACCCTGCG  
1441 -----+-----+-----+-----+-----+-----+-----+ 1500  
GGACCACCCGGAAAGGTAAAACCGGTTGTGCAAACACGGGTTTCTTTACGTCTGGGACGC  
L V G L S I L A N T F V P K E M Q T L R -  
TGTGGCTTTGGCATGTCTGGGAATCGGCTGTTCTGCTGCTACTTTTTCCAGTGTTGCTGT  
1501 -----+-----+-----+-----+-----+-----+-----+ 1560  
ACACCGAAACCGTACAGACCCTTAGCCGACAAGACGACGATGAAAAAGGTCACAACGACA  
V A L A C L G I G C S A A T F S S V A V -  
TCACTTCATTGAACTCATCCCCACTGTTCTCAGGGCAAGAGCTTCAGGAATAGATTTAAC  
1561 -----+-----+-----+-----+-----+-----+-----+ 1620  
AGTGAAGTAACTTGAGTAGGGGTGACAAGAGTCCCGTTCTCGAAGTCCTTATCTAAATTG  
H F I E L I P T V L R A R A S G I D L T -  
GGCTAGTAGGATTGGAGCAGCACTGGCTCCCCTCTTGATGACCTTAACGGTATTTTTTAC  
1621 -----+-----+-----+-----+-----+-----+-----+ 1680  
CCGATCATCCTAACCTCGTCGTGACCGAGGGGAGAACTACTGGAATTGCCATAAAAAATG  
A S R I G A A L A P L L M T L T V F F T -

FIGURE 6  
Sheet 3 of 5

CACTTTGCCATGGATCATTTATGGAATCTTCCCCATCATTTGGTGGCCTTATTGTCTTCCT  
1681 -----+-----+-----+-----+-----+-----+ 1740  
GTGAAACGGTACCTAGTAAATACCTTAGAAGGGGTAGTAACCACCGGAATAACAGAAGGA  
T L P W I I Y G I F P I I G G L I V F L -  
CCTACCAGAAACCAAGAATCTGCCTTTGCCTGACACCATCAAGGATGTGGAAAATCAAAA  
1741 -----+-----+-----+-----+-----+-----+ 1800  
GGATGGTCTTTGGTTCTTAGACGGAAACGGACTGTGGTAGTTCCTACACCTTTTAGTTTT  
L P E T K N L P L P D T I K D V E N Q K -  
AAAAAATCTCAAGGAAAAGGCATAAAAAATGATTGCTACACAAAAGTGACCAAATTTTAAG  
1801 -----+-----+-----+-----+-----+-----+ 1860  
TTTTTTAGAGTTCCTTTTCCGTATTTTACTAACGATGTGTTTTCACTGGTTTAAAATTC  
K N L K E K A \*  
AAGCCTTCATGAGCTGATTGGTGGGGAAATTCAGAAAAAAAATACAGGAAAAGAACACA  
1861 -----+-----+-----+-----+-----+-----+ 1920  
TTCGGAAGTACTCGACTAACCACCCCTTTAAGTCTTTTTTTTTTATGTCCTTTTCTTGTGT  
CCAGAAGGGTTTTTTTCCCTACAACCAGCAAGAACATATATTAGATACATGAATCTCAAT  
1921 -----+-----+-----+-----+-----+-----+ 1980  
GGTCTTCCCAAAAAAAGGGATGTTGGTCGTTCTTGTATATAATCTATGTAAGTTAGAGTTA  
TATAATTATGGCATTAATTTGCATTTTATTTCAAAATTAACCTGTGGGGACATGTAATCT  
1981 -----+-----+-----+-----+-----+-----+ 2040  
ATATTAATACCGTAATTAAACGTAAAATAAAGTTTAAATTGAACACCCCTGTACATTAGA  
CTTGAGCAATCTGATATTTTTTGGGAAGTCCTTTAAAAAGTTACAAATTTATCAATAAATT  
2041 -----+-----+-----+-----+-----+-----+ 2100  
GAACTCGTTAGACTATAAAAACCCTTCAGGAAATTTTCAATGTTTAAATAGTTATTTAA  
ACTAGTAGATAAGATGATTCAGAAACAAAGGAAAATCACAGAATTAGGATGTGGCTGGCT  
2101 -----+-----+-----+-----+-----+-----+ 2160  
TGATCATCTATTCTACTAAGTCTTTGTTTCCTTTTAGTGTCTTAATCCTACACCGACCGA  
TGGTGTATGAAGCACCATGTGATGAATTCATAAAGTTGCAAAAGTCAAAACAATACTGTA  
2161 -----+-----+-----+-----+-----+-----+ 2220  
ACCACATACTTCGTGGTACACTACTTAAGTATTTCAACGTTTTTCAGTTTTGTTATGACAT  
CATGCAACCAGAAATCAAATTAATCCAGAAATAGAGACCTATATAAATGCATTTAATAC  
2221 -----+-----+-----+-----+-----+-----+ 2280  
GTACGTTGGTCTTTAGTTTAAATTTAGGTCTTTATCTCTGGATATATTTACGTAAATTATG  
ATGATACTTTTGACATATTAAGCCATTGGAAAACGGAAGGATTAGATACTTAAATAACAT  
2281 -----+-----+-----+-----+-----+-----+ 2340  
TACTATGAAAACGTGATAATTCGGTAACCTTTTGCCTTCCTAATCTATGAATTTATTGTA  
TGCTATCTCTTTGTAAATACAGTCACTAAATGATGTTAGTTACTTTTCCATGGTGGAAAT  
2341 -----+-----+-----+-----+-----+-----+ 2400  
ACGATAGAGAAACATTTATGTCAGTGATTTACTACAATCAATGAAAAGGTACCACCTTAA

FIGURE 6  
Sheet 4 of 5

TTAATTACTTTTCTTTGTAATTTTCTCTCTGTATATTTTAAACAAATAGCTGGTATAG  
2401 -----+-----+-----+-----+-----+-----+ 2460  
AATTAATGAAAAAGAAACATTAAAAAGAGAGACATATAAAATTTGTTTATCGACCATATC  
TTTACAATATTATAAAGATATTGTTCAAATTGAAGGGCAAAGGCCAGGTTTCAGCAATTTT  
2461 -----+-----+-----+-----+-----+-----+ 2520  
AAATGTTATAATATTTCTATAACAAGTTTAACTTCCCGTTTCCGGTCCAAGTCGTTAAAA  
CAAACGTATGTACATTTAATAAAATAACTATAAATTAAAAAATTATATTTCAAATGATG  
2521 -----+-----+-----+-----+-----+-----+ 2580  
GTTTGACATACATGTAAATTATTTTATTGATATTTAATTTTAAATATAAAGTTTACTAC  
TGACTAATAAATGAAAGTACATATAGTAGTAAAGTAATTTTCAGGCAAACCTATATAACCA  
2581 -----+-----+-----+-----+-----+-----+ 2640  
ACTGATTATTTACTTTTCATGTATATCATCATTTTCATTAAAGTCCGTTTGGATATATTGGT  
AAATATAAACTTTCATTTTAAACAGCAAAAAAAAAAAAAAAAAAAAA  
2641 -----+-----+-----+-----+-----+----- 2684  
TTTATATTTGAAAGTAAATTTGTCGTTTTTTTTTTTTTTTTTTT

FIGURE 6  
Sheet 5 of 5

# Transport of pAH by OAT 1 in HeLa

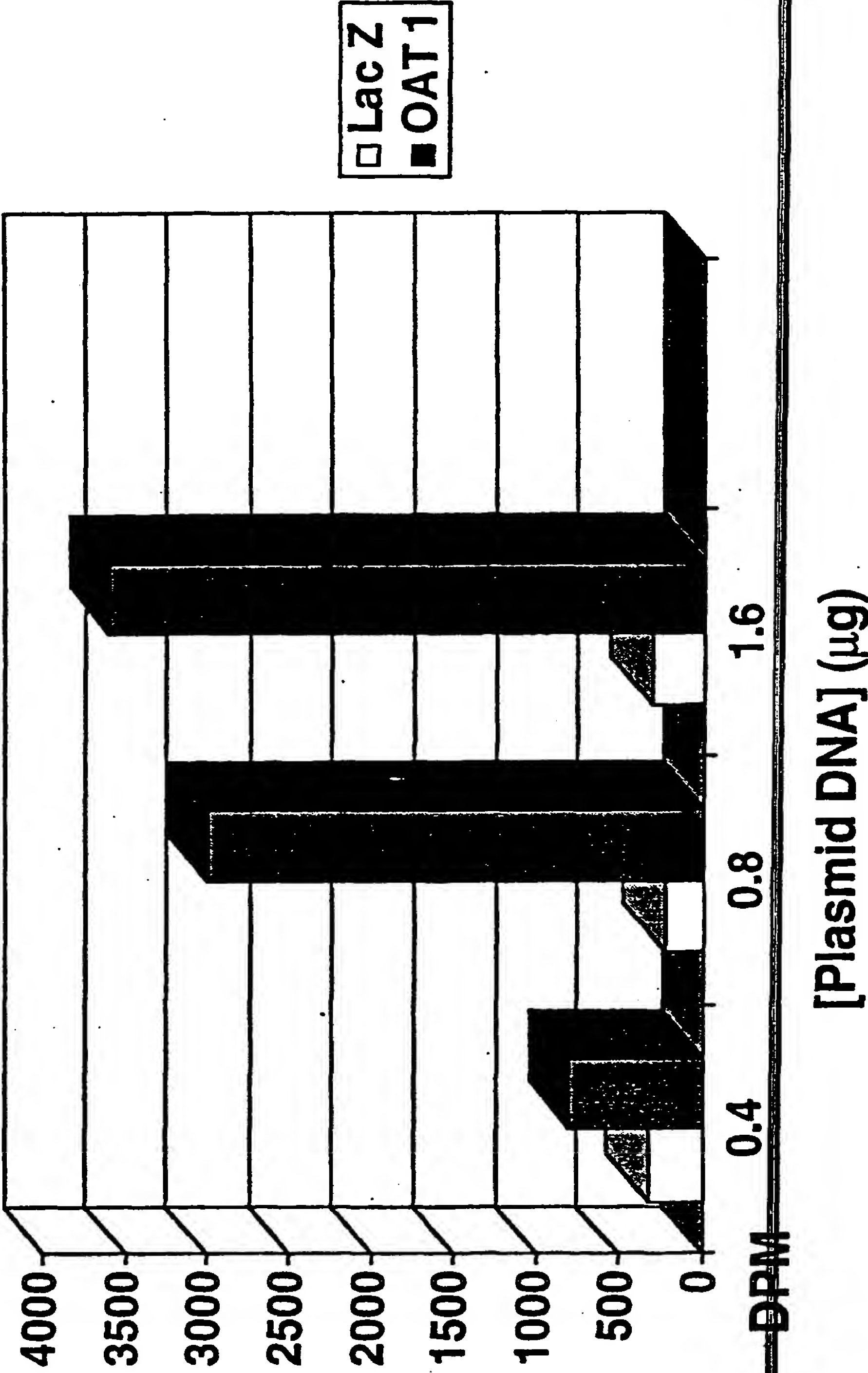


FIGURE 7

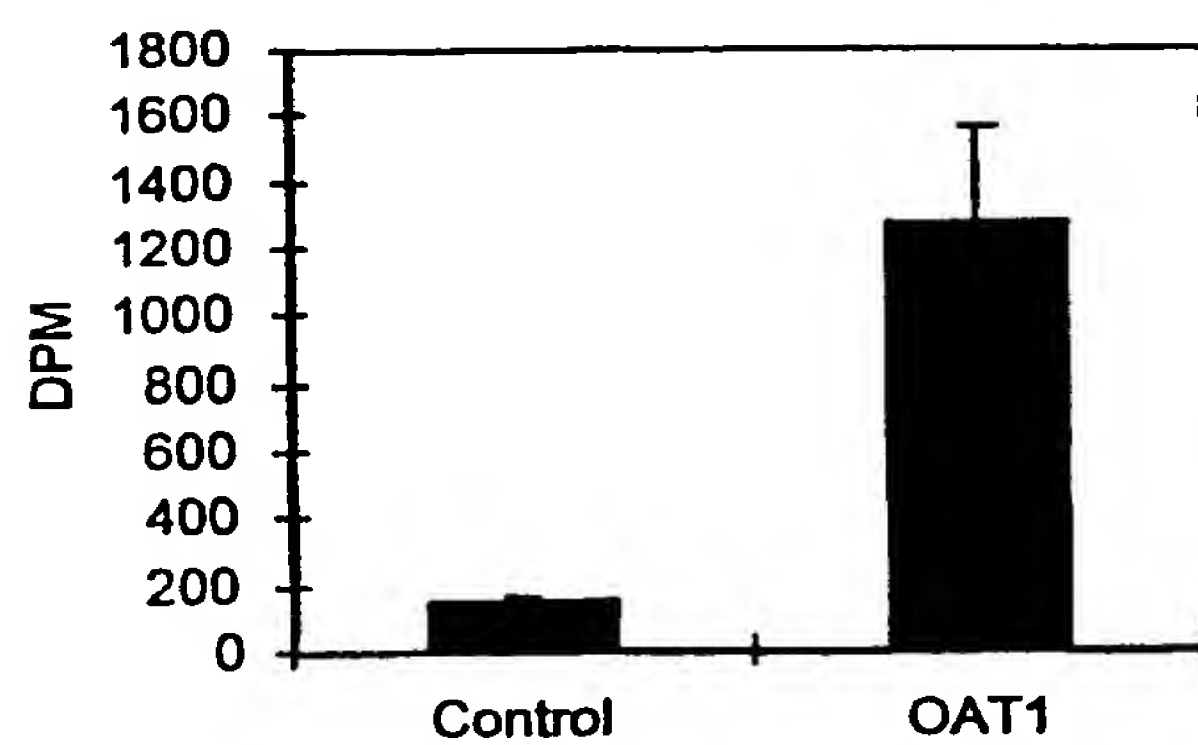


FIGURE 8



# PAH Uptake by OAT1 EcR Cell Line

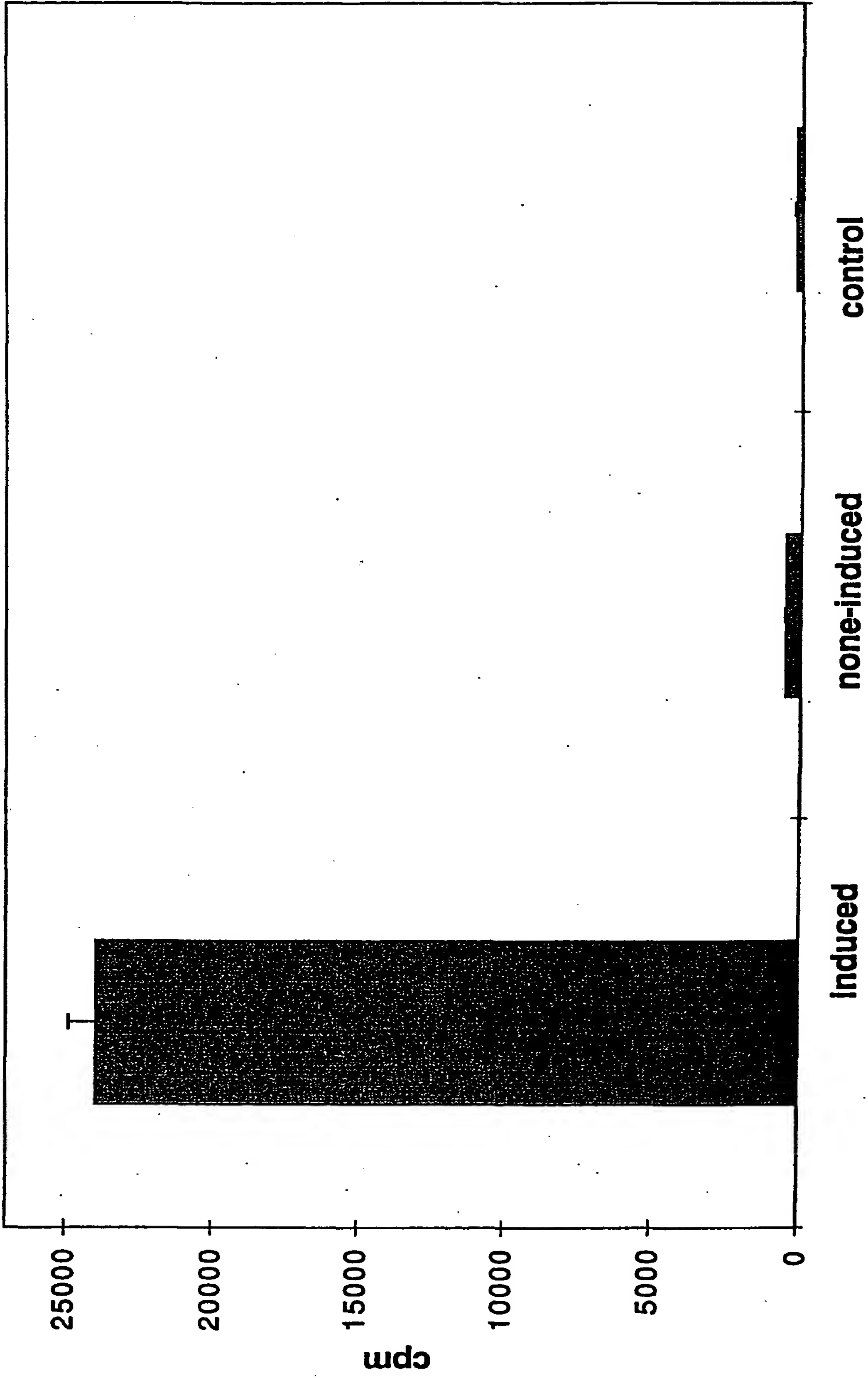


FIGURE 9

# PAH Uptake by OAT2 EcR Cell Line

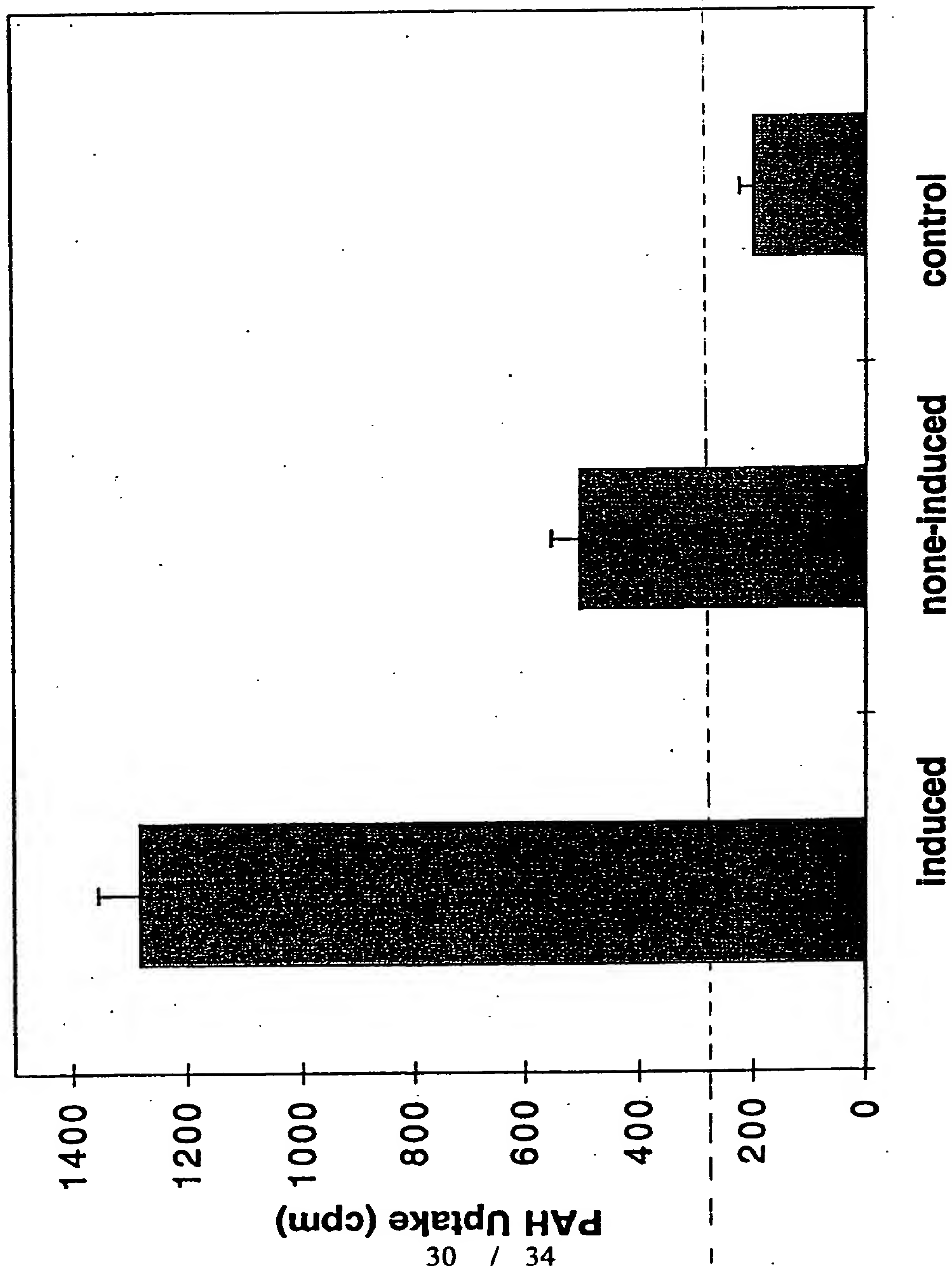


FIGURE 10

## PAH Transport by EcR293-OAT2A

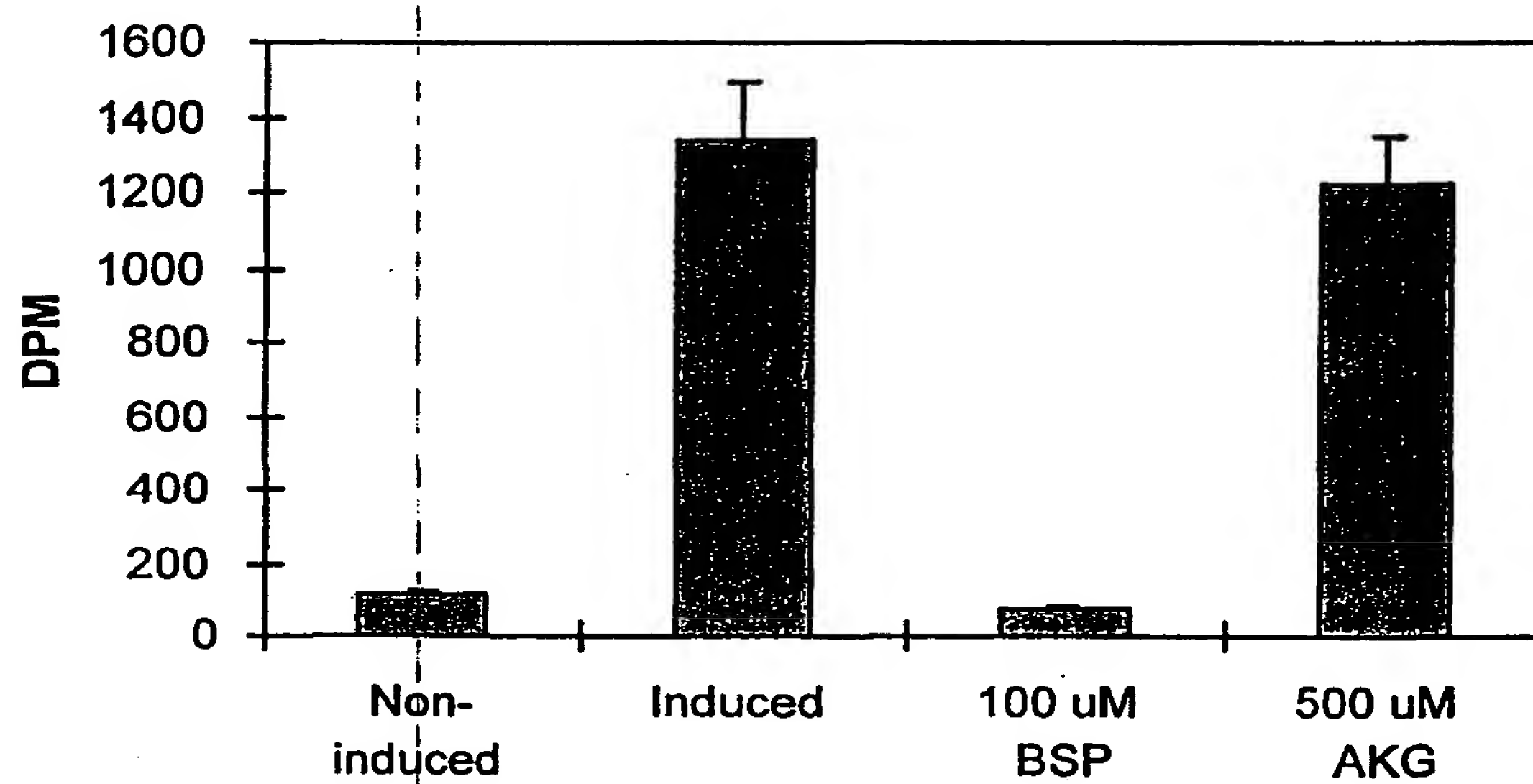


FIGURE 11

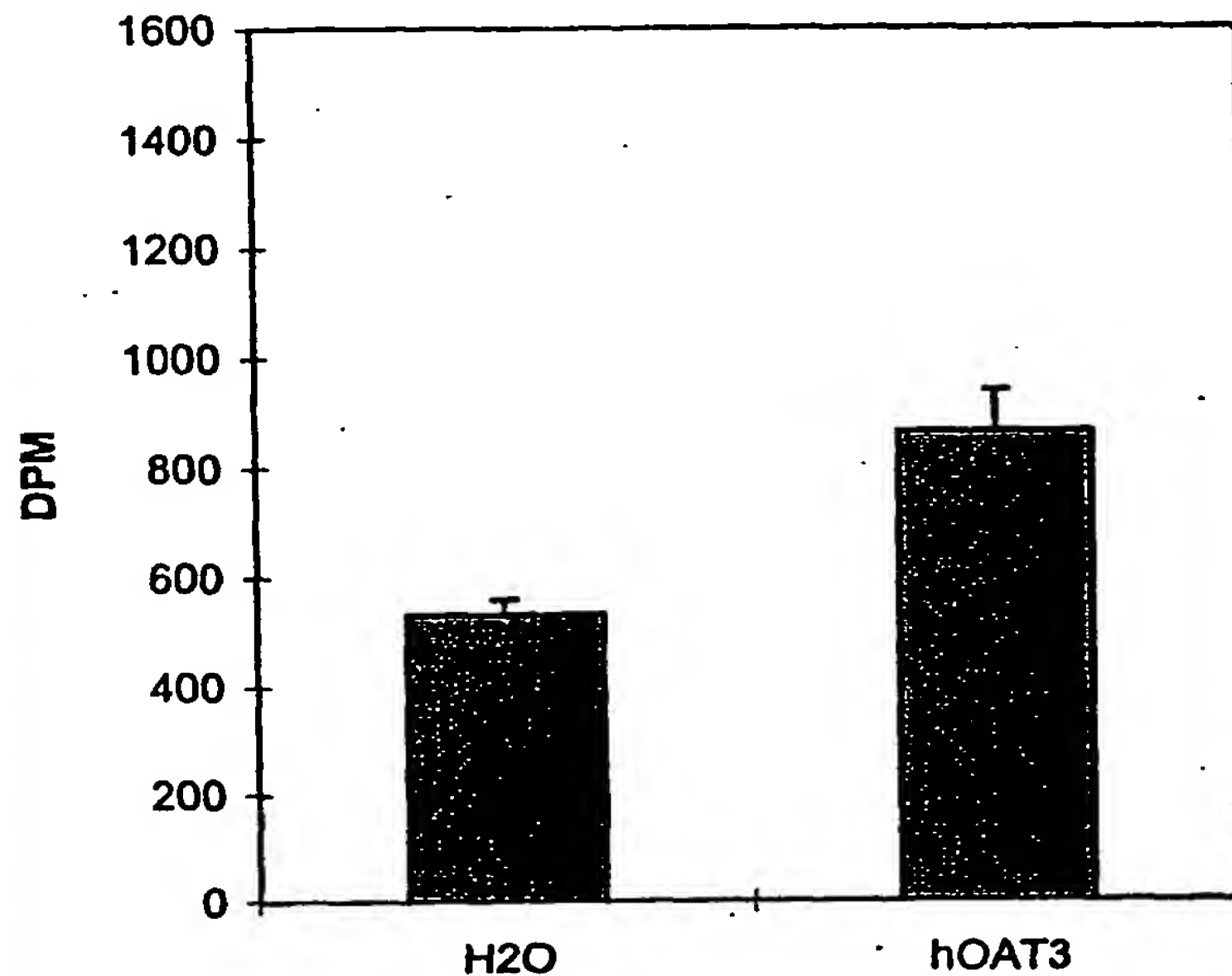


Figure 12: Expression of hOAT3 in *Xenopus* oocytes. Oocytes were injected with either water or hOAT3 complementary RNA and then assayed for uptake of labeled PAH.

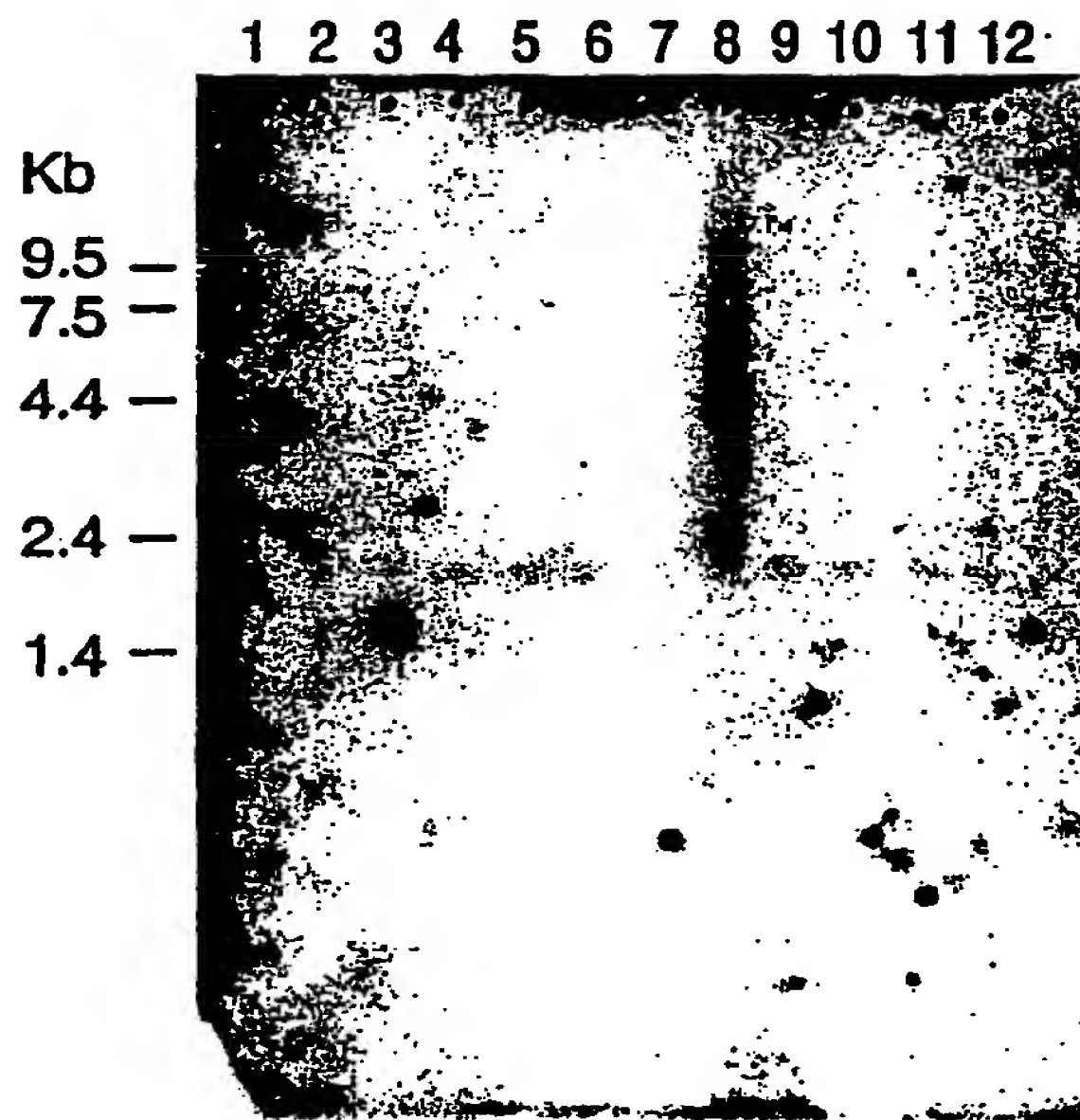
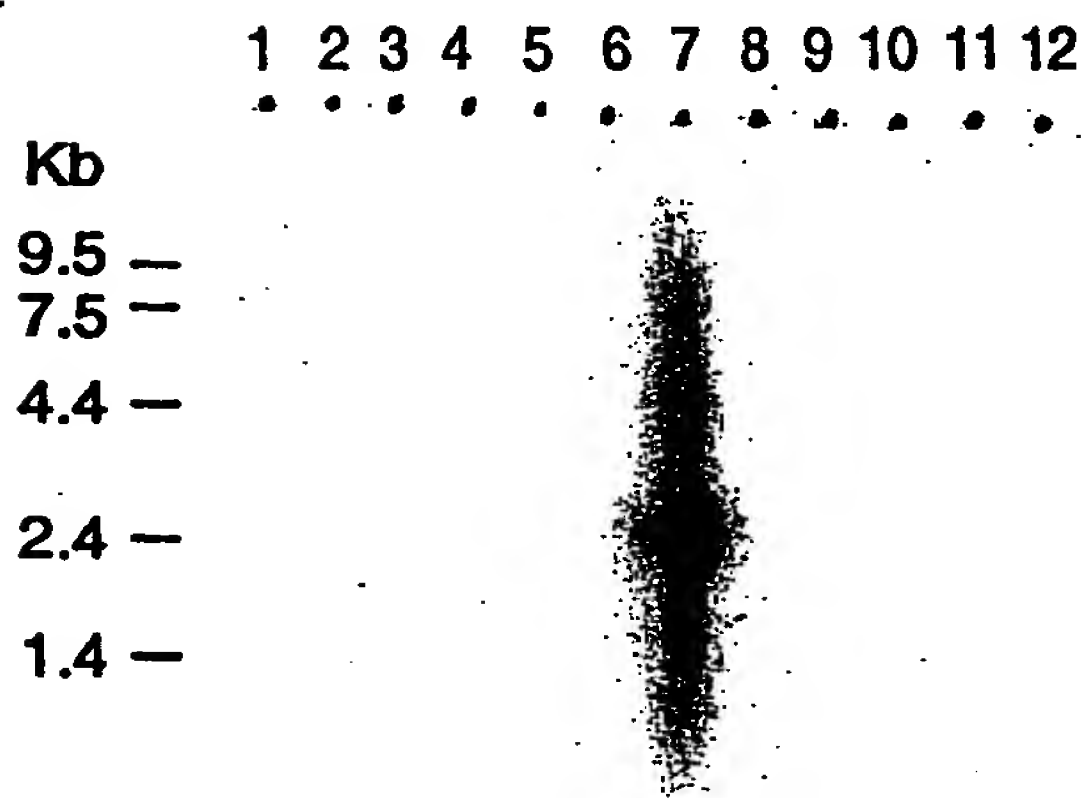
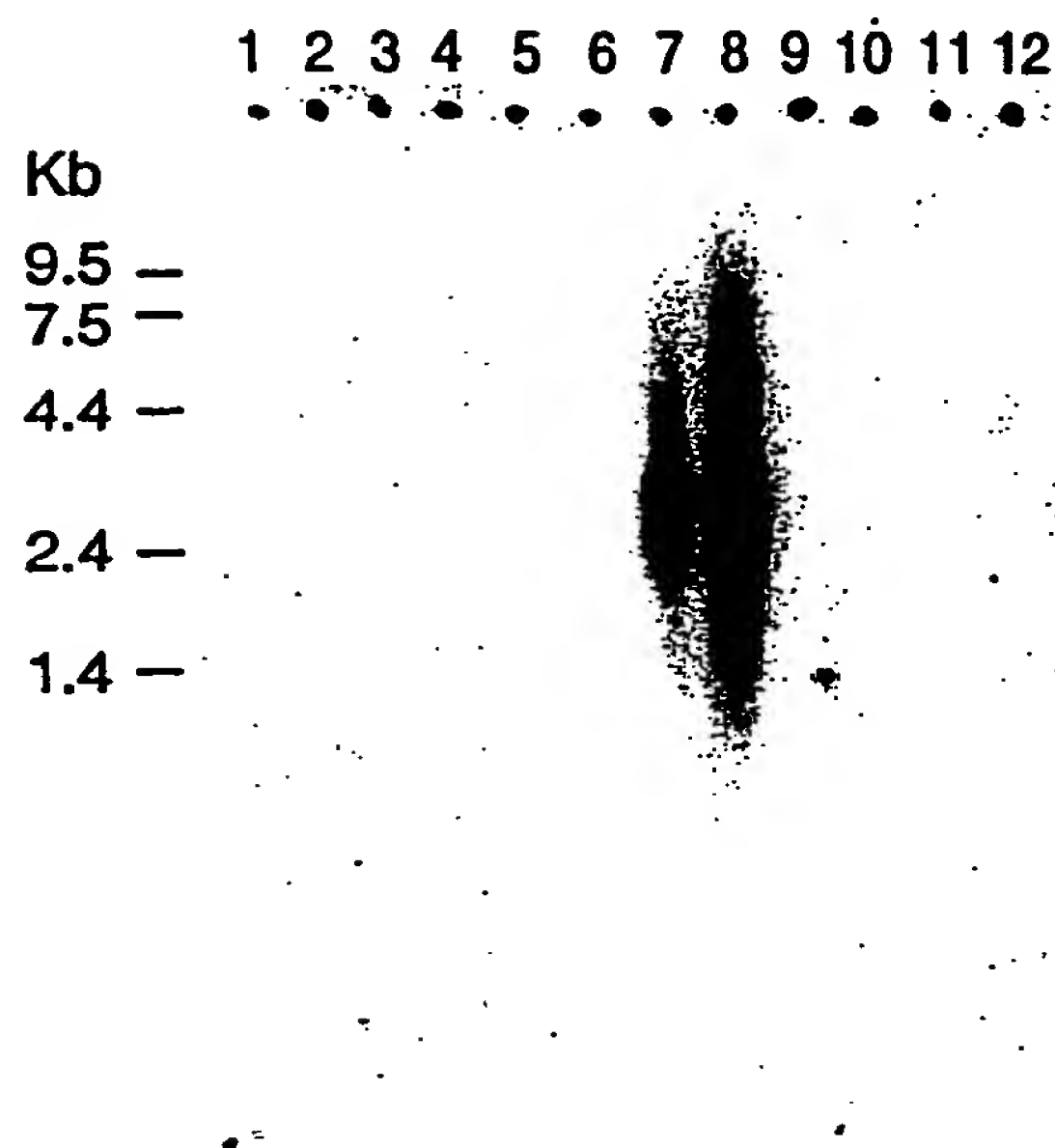
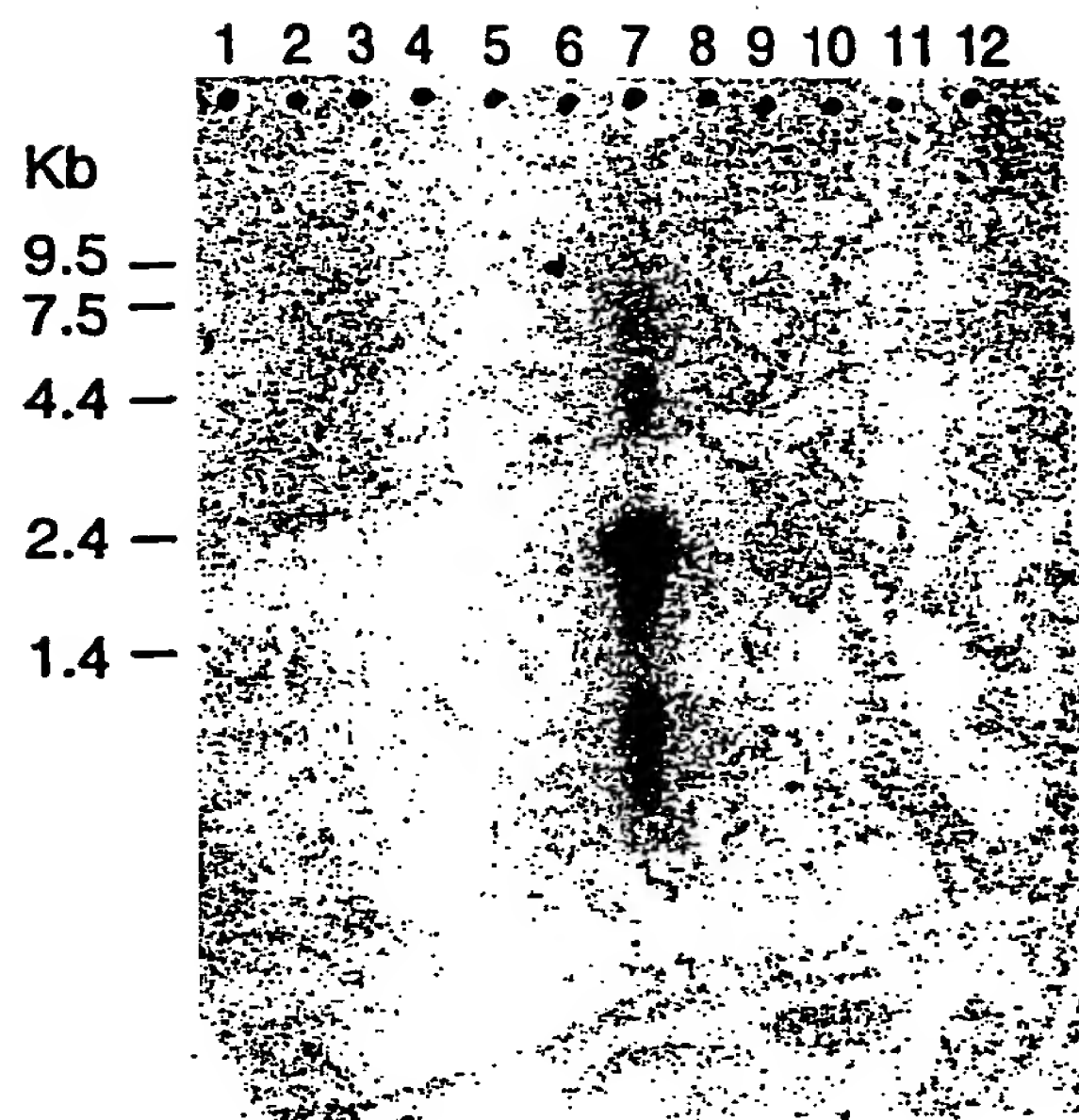


FIGURE 13  
Sheet 1 of 2



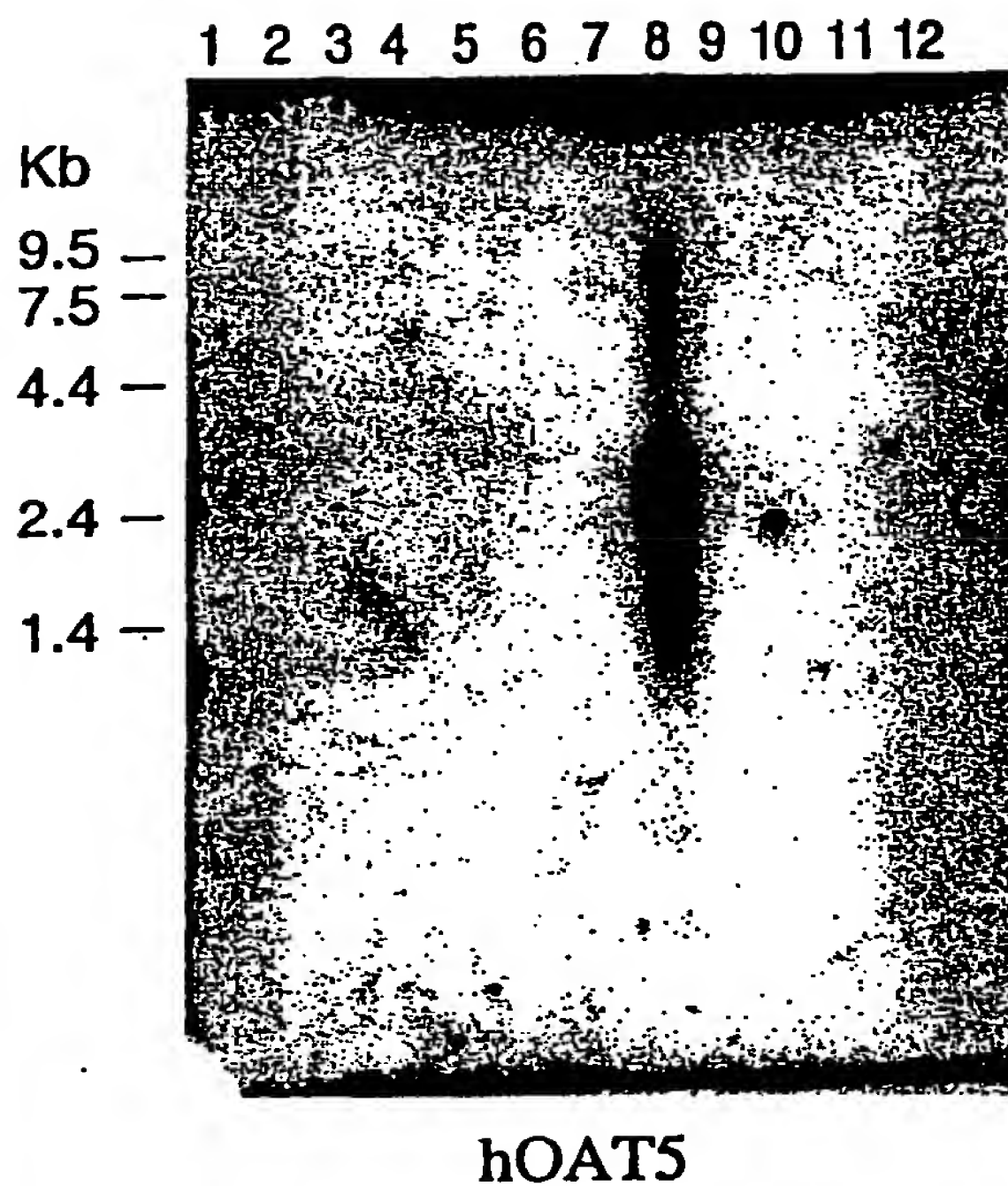


Figure 13: Human Multiple-tissue Northern Blots (Clontech) were hybridized with indicated hOAT cDNAs. Lane numbers corresponds to RNAs extracted from following human tissues:

- |                    |                                  |
|--------------------|----------------------------------|
| 1. brain           | 7. kidney                        |
| 2. heart           | 8. liver                         |
| 3. skeletal muscle | 9. small intestine               |
| 4. colon           | 10. placenta                     |
| 5. thymus          | 11. lung                         |
| 6. spleen          | 12. peripheral blood leukocytes. |

FIGURE 13  
Sheet 2 of 2

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International Bureau**



**(43) International Publication Date**  
**18 January 2001 (18.01.2001)**

**(10) International Publication Number**  
**WO 01/04283 A3**

## PCT

- |  |   |  |  |
|--|---|--|--|
| <b>(51) International Patent Classification<sup>7</sup>:</b> | <b>C12N 15/12, C07K 14/705, C12Q 1/68, C07K 16/28, G01N 33/68</b>                               | <b>(72) Inventor:</b>                            | <b>SUN, William; 12552 San Bruno Cove, San Diego, CA 92130 (US).</b>   |
| <b>(21) International Application Number:</b>                | <b>PCT/US00/18980</b>   | <b>(74) Agents:</b>                              | <b>WOLFF, Jessica, R. et al.; Brobeck, Phleger &amp; Harrison, 12390 El Camino Real, San Diego, CA 92130 (US).</b>   |
| <b>(22) International Filing Date:</b>                       | <b>12 July 2000 (12.07.2000)</b>  | <b>(81) Designated States (<i>national</i>):</b> | <b>AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.</b> |
| <b>(25) Filing Language:</b>                                 | <b>English</b>  | <b>(84) Designated States (<i>regional</i>):</b> | <b>ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,</b>   |
| <b>(26) Publication Language:</b>                            | <b>English</b>  |  |  |
| <b>(30) Priority Data:</b>                                   | <b>60/143,771 12 July 1999 (12.07.1999) US</b>  |  |  |
| <b>(71) Applicant:</b>                                       | <b>METABASIS THERAPEUTICS, INC. [US/US]; 9390 Towne Centre Drive, San Diego, CA 92121 (US).</b> |  |  |

*[Continued on next page]*

**(54) Title: ORGANIC ANION TRANSPORTER GENES AND PROTEINS**

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C

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**D**

**(57) Abstract:** This invention relates to novel human organic anion transporter polypeptides and genes encoding such polypeptides. The invention is directed towards the isolation, characterization and use of human organic anion transporter polypeptides for pharmacological screening of substrates, analogues of substrates, and modulators of human organic anion proteins. This invention also provides cell cultures, which stably contain nucleic acid sequences encoding human organic anion transport polypeptides. Such cell lines are useful for screening compounds *in vitro* to identify and characterize modulators, inhibitors and substrates of human organic anion transporter polypeptides.



IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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## INTERNATIONAL SEARCH REPORT

International Application No

PL./US 00/18980

|  |  |  |
|--|--|--|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC 7 C12N15/12 C07K14/705 C12Q1/68 C07K16/28 G01N33/68  |  |  |
| According to International Patent Classification (IPC) or to both national classification and IPC  |  |  |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC 7 C07K C12N  |  |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  |  |  |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)<br>EPO-Internal, EMBL, BIOSIS   |  |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>  |  |  |
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |
| X  | DATABASE EMBL [Online]<br>Accession number AF097518,<br>7 June 1999 (1999-06-07)<br>KIM R.B. ET AL.: "homo sapiens<br>liver-specific transporter (NLT) mRNA,<br>complete cds."<br>XP002156221<br>the whole document  | 1-5,9,<br>10,<br>14-16,<br>20,24-54                                |
| X  | KUSUHARA HIROYUKI ET AL: "Molecular<br>cloning and characterization of a new<br>multispecific organic anion transporter<br>from rat brain."<br>JOURNAL OF BIOLOGICAL CHEMISTRY,<br>vol. 274, no. 19, 7 May 1999 (1999-05-07),<br>pages 13675-13680, XP002156219<br>ISSN: 0021-9258<br>the whole document | 1-6,<br>9-11,<br>14-17,<br>20,21,<br>24-54                         |
| - / - -  |  |  |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.  |  |  |
| <b>* Special categories of cited documents :</b><br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier document but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed<br>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br>"&" document member of the same patent family |  |  |
| Date of the actual completion of the international search<br><br>5 January 2001  |  | Date of mailing of the international search report<br><br>06.02.01 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016   |  | Authorized officer<br><br>Gurdjian, D                              |

# INTERNATIONAL SEARCH REPORT

International Application No  
PL./US 00/18980

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |  |                           |
|--|--|---------------------------|
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.     |
| X  | <p>DATABASE EMBL [Online]<br/>Accession number AI016020,<br/>18 June 1998 (1998-06-18)<br/>ROBERT STRAUSBERG: " similar to TR:Q63314<br/>Q63314 LIVER-SPECIFIC TRANSPORT PROTEIN"<br/>XP002156222<br/>the whole document</p>   | 1-5,9,<br>10,<br>14-16,20 |
| A  | <p>---<br/>WO 98 53064 A (HOSOYAMADA MAKOTO ;TANABE<br/>SEIYAKU CO (JP); ENDOU HITOSHI (JP); SEK)<br/>26 November 1998 (1998-11-26)<br/>abstract<br/>page 22-32</p>  | 1-54                      |
| A  | <p>---<br/>SIMONSON GREGG D ET AL: "Molecular<br/>cloning and characterization of a novel<br/>liver-specific transport protein."<br/>JOURNAL OF CELL SCIENCE,<br/>vol. 107, no. 4, 1994, pages 1065-1072,<br/>XP000971769<br/>ISSN: 0021-9533<br/>the whole document<br/>-----</p> | 1-54                      |



# INTERNATIONAL SEARCH REPORT

national application No.  
PCT/US 00/18980

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3 ,14, 24-54 partly and 4,5,9,10,15,16,20

human organic anion transporter OAT2a and OAT2b with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

2. Claims: 1-3 ,14, 24-54 partly and 6,11,17,21 partly

human organic anion transporter OAT3 with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

3. Claims: 1-3 ,14, 24-54 partly and 7,12,18,22 partly

human organic anion transporter OAT4 with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

4. Claims: 1-3 ,14, 24-54 partly and 8,13,19,23 partly

human organic anion transporter OAT5 with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

**Information on patent family members**

PL./US 00/18980

Patent document  
cited in search report

Publication  
date

Patent family member(s)

Publication date

WO 9853064

A

26-11-1998

AU

7238498 A

11-12-1998

JP

11032786 A

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## CORRECTED VERSION

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|--|---|--|--|
| <b>(51) International Patent Classification<sup>7</sup>:</b> | <b>C12N 15/12, C07K 14/705, C12Q 1/68, C07K 16/28, G01N 33/68</b>                               | <b>(72) Inventor:</b>                            | <b>SUN, William; 12552 San Bruno Cove, San Diego, CA 92130 (US).</b>   |
| <b>(21) International Application Number:</b>                | <b>PCT/US00/18980</b>   | <b>(74) Agents:</b>                              | <b>WOLFF, Jessica, R. et al.; Brobeck, Phleger &amp; Harrison, 12390 El Camino Real, San Diego, CA 92130 (US).</b>   |
| <b>(22) International Filing Date:</b>                       | <b>12 July 2000 (12.07.2000)</b>  | <b>(81) Designated States (<i>national</i>):</b> | <b>AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.</b> |
| <b>(25) Filing Language:</b>                                 | <b>English</b>  | <b>(84) Designated States (<i>regional</i>):</b> | <b>ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian</b>   |
| <b>(26) Publication Language:</b>                            | <b>English</b>  |  |  |
| <b>(30) Priority Data:</b>                                   | <b>60/143,771 12 July 1999 (12.07.1999) US</b>  |  |  |
| <b>(71) Applicant:</b>                                       | <b>METABASIS THERAPEUTICS, INC. [US/US]; 9390 Towne Centre Drive, San Diego, CA 92121 (US).</b> |  |  |

*[Continued on next page]*

- (54) Title: ORGANIC ANION TRANSPORTER GENES AND PROTEINS**

**(57) Abstract:** This invention relates to novel human organic anion transporter polypeptides and genes encoding such polypeptides. The invention is directed towards the isolation, characterization and use of human organic anion transporter polypeptides for pharmacological screening of substrates, analogues of substrates, and modulators of human organic anion proteins. This invention also provides cell cultures, which stably contain nucleic acid sequences encoding human organic anion transport polypeptides. Such cell lines are useful for screening compounds *in vitro* to identify and characterize modulators, inhibitors and substrates of human organic anion transporter polypeptides.

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A

[illegible]

**E**

[illegible]

C

[illegible]

**D**

**WO 01/04283 A3**



patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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## ORGANIC ANION TRANSPORTER GENES AND PROTEINS

### Related Applications

This application claims the benefit of U.S. Serial No. 60/143,771, entitled  
5 "Organic Anion Transporter Genes and Proteins," which was filed July 12, 1999 and  
which is incorporated by reference herein in its entirety, including figures.

### Field of the Invention

The present invention relates to specific proteins as well as recombinant versions  
10 of these proteins which are organic anion transporters. These proteins include human  
membrane proteins preferentially found in kidney and liver cells. The present invention  
also relates to nucleotide sequences encoding these novel organic anion transporters. In  
another aspect, the present invention relates to methods for using these proteins as *in vitro*  
screening agents to identify substrates for the proteins and inhibitors which block transport  
15 activity. In yet another aspect, these proteins may be used in *in vitro* assays to predict  
drug pharmacokinetics, drug distribution and drug toxicity. The invention also discloses  
antibodies that specifically bind to these proteins and which may be used in diagnostic  
assays.

### 20 Background of the Invention

The following describes certain relevant art, none of which is admitted to be prior  
art to the inventions described herein.

The liver and kidney are two organs that can extract a variety of organic anions  
from circulation, including endogenous compounds such as bile acids and bilirubin and  
25 xenobiotics such as sulfobromophthalein (BSP) and *p*-aminohippurate (PAH). The liver  
and kidney thus have the critical functions of bile secretion, detoxification, and drug  
elimination. Since the cell membrane represents a hydrophobic barrier that prevents influx  
of charged or hydrophilic molecules, hepatocytes and kidney epithelial cells express  
proteins on their basolateral membranes to facilitate transport of organic anions into the  
30 cell. The liver and kidney contain membrane proteins that transport very specific  
compounds such as taurocholic acid or prostaglandins. However, they also have proteins  
that exhibit a wider substrate specificity. Recently, transporters of the latter type have  
been cloned from rat liver and kidney. One such protein is called the organic anion

transporting polypeptide, oatp (Jacquemin et al. (1994) *PNAS* 91:133-37). This protein belongs to a family of related transporters, members of which consists of oatp1, oatp2, oatp3, and prostaglandin transporters. These proteins share homology at the amino acid level (Noe et al. (1997) *PNAS* 94: 10346-50; Kanai et al. (1995) *Science* 268: 866-69; Abe  
5 et al. (1998) *J. Biol. Chem.* 273: 22395-401). When expressed in *Xenopus* oocytes or in mammalian cells, members of the oatp family were shown to transport many types of organic anions including taurocholic acid, BSP, and conjugated steroid hormones. The related prostaglandin transporters show high affinity transport of prostaglandins.

Shortly after the cloning of oatp1, members of another organic anion transporter  
10 family, OAT1 and OAT2, were isolated from rat kidney and liver, respectively (Sekine et al. (1997) *J. Biol. Chem* 272: 18526-29; Sekine et al. (1998) *FEBS Letters* 429: 179-82). Rat OAT1 and OAT2 are homologous to each other but show no significant homology to members of the oatp family. However, they show some homology to the family of organic  
15 cation transporters (OCTs), suggesting that these two families of proteins share a common origin. Rat OAT1 and OAT2 can transport many different organic anions. OAT1, when expressed in oocytes, can transport *p*-aminohippurate (PAH), methotrexate, and glutarate. OAT1 is most likely the molecular entity responsible for the classical *p*-aminohippurate/ $\alpha$ -ketoglutarate exchanger found in the kidney proximal tubule. OAT2, expressed  
predominantly in the liver, has been shown to transport organic anions such as salicylate,  
20 methotrexate, and  $\alpha$ -ketoglutarate. Therefore, OAT1 and OAT2 belong to yet another family of multi-specific organic anion transporters distinct from the oatp family.

### Summary of the Invention

The present invention is directed to a group of polypeptides, preferentially  
25 expressed in the liver and kidneys of humans and which have activity as organic anion transporters. We refer to these polypeptides as "human organic anion transporters" or "hOAT polypeptides". These proteins and their properties are described in detail below.

The invention also concerns, nucleic acids encoding hOAT polypeptides, cells, tissues and animals containing such nucleic acids, antibodies to the polypeptides, assays  
30 utilizing the polypeptides, and methods relating to all of the foregoing.

A first aspect of the invention features an isolated, enriched, or purified nucleic acid molecule encoding an hOAT polypeptide or encoding a fragment of an hOAT polypeptide.

In preferred embodiments the isolated nucleic acid comprises a nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof, a nucleic acid sequence that hybridizes to the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof.

The nucleic acid may be isolated from a natural source by cDNA cloning or subtractive hybridization; the natural source may be mammalian (human) blood, semen, or tissue and the nucleic acid may be synthesized by the triester or other method or by using an automated DNA synthesizer. Preferably, the nucleic acid is isolated from mammalian kidney or brain, most preferably from liver.

Individual clones isolated from a cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones can be obtained directly from total DNA or from total RNA. The cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately  $10^6$ -fold purification of the native message. Thus, purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

In another preferred embodiment, the nucleic acid molecules of the invention comprise nucleotide sequences that (a) have the nucleic acid sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof, or encode polypeptides having the full-length amino acid sequences set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof; (b) is the complement of the nucleotide sequence of (a); (c) hybridizes under highly stringent conditions to the nucleotide molecules of (a) and encodes a naturally occurring hOAT polypeptide; (d) encodes an hOAT polypeptide having the full-length amino acid sequence of the sequence set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12 except that it lacks one or more of the domains selected from the group

consisting of an extracellular domain, a transmembrane domain, and an intracellular domain; or (e) is the complement of the nucleotide sequence of (d).

The nucleic acid molecules of the invention are isolated, enriched, or purified preferably from a mammal, more preferably from a human.

5 Hybridization techniques may be used to isolate a nucleic acid molecule of interest. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Stringency is controlled by varying salt or denaturant concentrations. Highly stringent conditions may mean conditions that are at least as stringent as the following: hybridization in 50% formamide, 5x SSC, 50 mM  $\text{NaH}_2\text{PO}_4$ ,  
10 pH 6.8, 0.5% SDS, 0.1 mg/mL sonicated salmon sperm DNA, and 5x Denhart solution at 42 °C overnight; washing with 2x SSC, 0.1% SDS at 45 °C; and washing with 0.2x SSC, 0.1% SDS at 45 °C. Those skilled in the art will recognize how such conditions can be varied to vary specificity and selectivity.

Particularly preferred embodiments of this aspect of the invention are naturally or  
15 non-naturally occurring variants of the nucleic acids of the invention. Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or  
20 non-conservative amino acid substitutions, deletions or additions. Such nucleic acid molecules are identifiable as being able to hybridize to or which are at least about 60-65% percent identical, preferably at least about 70-75% percent identical, more preferably at least about 80-83% percent identical, and even more preferably at least about 87-95% percent identical to the nucleic acid molecules shown in SEQ ID NO:1, SEQ ID NO:2,  
25 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

Among the particularly preferred embodiments of the invention in this regard are nucleic acid molecules encoding polypeptides having the amino acid sequences set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12; variants, analogs, derivatives and fragments thereof, and fragments of the variants,  
30 analogs and derivatives.

Further particularly preferred in this regard are nucleic acid molecules encoding OAT variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence for the hOAT polypeptides of SEQ ID

NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the OAT polypeptides. Also especially preferred in this regard are conservative substitutions. In one embodiment, the polypeptide encoded by the nucleic acid molecule is at least about 30-35%, preferably at least about 40-45%, more preferably at least about 50-55%, even more preferably at least about 60-65%, yet more preferably at least about 70-75%, still more preferably at least about 80-85%, and most preferably at least about 90-95% or more identical to the amino acid sequence of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

In still another embodiment, the nucleic acid molecule encodes a naturally occurring variant of the polypeptide of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12 and hybridizes under stringent conditions to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, respectively.

Further preferred embodiments are variants of the herein described nucleic acid molecules in which the transporting activity of the polypeptides is altered. Such alterations can result in either increased, or decreased transport of substrates recognized by hOAT polypeptides. Further, variants can be made that result in OAT polypeptides which are no longer responsive to cellular signals, for example, protein kinase C deactivation of transport.

Especially preferred are nucleic acid molecules encoding OATs which are expressed on the basolateral (sinusoid) membrane of hepatocyte cells.

Included in the invention are all possible nucleic acid sequences that encode the amino acid sequences of the hOAT polypeptides of the invention. As it is recognized that alternate codons will encode the same amino acid for most amino acids due to the degeneracy of the genetic code, the sequences of this aspect includes nucleic acid sequences utilizing such alternate codon usage for one or more codons of a coding sequence. For example, all four nucleic acid sequences GCT, GCC, GCA, and GCG encode the amino acid, alanine. Therefore, if for an amino acid there exists an average of three codons, a polypeptide of 100 amino acids in length will, on average, be encoded by  $3^{100}$ , or  $5 \times 10^{47}$ , nucleic acid sequences. Thus, a nucleic acid sequence can be modified



(e.g., a nucleic acid sequence from an hOAT as specified above) to form a second nucleic acid sequence encoding the same polypeptide as encoded by the first nucleic acid sequence using routine procedures and without undue experimentation. Thus, all possible nucleic acid sequences that encode the amino acid sequences of the hOAT polypeptides of the invention are included in the present invention and are described, as if all were written out in full, taking into account the codon usage.

The alternate codon descriptions are available in common textbooks, for example, Stryer, BIOCHEMISTRY 3<sup>rd</sup> ed., and Lehninger, BIOCHEMISTRY 3<sup>rd</sup> ed. Codon preference tables for various types of organisms are available in the literature. Because of the number of sequence variations involving alternate codon usage, for the sake of brevity, individual sequences are not separately listed herein. Instead the alternate sequences are described by reference to the natural sequence with replacement of one or more (up to all) of the degenerate codons with alternate codons from the alternate codon table, preferably with selection according to preferred codon usage for the normal host organism or a host organism in which a sequence is intended to be expressed. Those skilled in the art also understand how to alter the alternate codons to be used for expression in organisms where certain codons code differently than shown in the "universal" codon table.

In a second aspect, the invention relates to a nucleic acid vector comprising a nucleic acid molecule encoding an hOAT polypeptide and a promoter element effective to initiate transcription in a host cell.

Those skilled in the art would recognize that a nucleic acid vector can contain many other nucleic acid elements besides the promoter element and the hOAT nucleic acid molecule. These other nucleic acid elements include, but are not limited to, origins of replication, ribosomal binding sites, nucleic acid sequences encoding drug resistance enzymes or amino acid metabolic enzymes, and nucleic acid sequences encoding secretion signals, periplasm or peroxisome localization signals, or signals useful for polypeptide purification.

A circular double stranded nucleic acid molecule can be cut and thereby linearized upon treatment with restriction enzymes. An assortment of vectors, restriction enzymes, and the knowledge of the nucleotide sequences that the restriction enzymes operate upon are readily available to those skilled in the art. A nucleic acid molecule of the invention can be inserted into a vector by cutting the vector with restriction enzymes and ligating the two pieces together.



Many techniques are available to those skilled in the art to facilitate transformation or transfection of the expression construct into a prokaryotic or eukaryotic organism. These methods involve a variety of techniques, such as treating the cells with high concentrations of salt, an electric field, or detergent, to render the host cell outer  
5 membrane or wall permeable to nucleic acid molecules of interest.

In a third aspect, the invention features a nucleic acid probe for the detection of a nucleic acid encoding an hOAT polypeptide, fragment or analogue in a sample. The nucleic acid probe contains nucleotides that will hybridize specifically to a sequence of at least 14 contiguous nucleotides set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3,  
10 SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a functional derivative thereof. The probe is preferably at least 14 or more bases in length and selected to hybridize specifically to a unique region of an hOAT encoding nucleic acid.

In preferred embodiments, the nucleic acid probe hybridizes to at least 14 nucleotides of a nucleic acid encoding the full-length sequence set forth in SEQ ID NO:7,  
15 SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Under highly stringent hybridization conditions, only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 1 or 2  
20 mismatches out of 20 contiguous nucleotides.

In another preferred embodiment, the nucleic acid is an isolated conserved or unique region, for example, those useful for the design of hybridization probes to facilitate identification and cloning of additional polypeptides, or for the design of PCR probes to facilitate amplification or cloning of additional polypeptides. Such a nucleic acid may  
25 contain additional sequences in addition to the conserved or unique region, preferably 10-20 additional nucleotides on each side of the conserved or unique region, more preferably 30-40 additional nucleotides, and most preferably 75-100 additional nucleotides.

Methods for using the probes include detecting the presence or amount of hOAT polypeptide RNA in a sample by contacting the sample with a nucleic acid probe under  
30 conditions such that hybridization occurs and detecting the presence or amount of the probe bound to hOAT polypeptide RNA. The nucleic acid duplex formed between the probe and a nucleic acid sequence coding for an hOAT polypeptide may be used in the identification of the sequence of the nucleic acid detected (for example see, Nelson et al.,

in Nonisotopic DNA Probe Techniques, p. 275 Academic Press, San Diego (Kricka, ed., 1992) hereby incorporated by reference herein in its entirety, including any drawings). Kits for performing such methods may be constructed to include a container having disposed therein a nucleic acid probe.

5           Another feature of the invention is a nucleic acid molecule as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or fragments thereof, comprising one or more regions that encode an hOAT polypeptide or an hOAT domain polypeptide, where the hOAT polypeptide or the hOAT domain polypeptide is fused to a non-hOAT polypeptide or amino-terminal tag. Possible non-  
10 hOAT polypeptide or amino-terminal tag fusion partners include, for example, but are not limited to, glutathione-S-transferase (GST)-fusion proteins, green fluorescent protein (GFP) and fusions with histidine residues as described by Janknecht et al., 1991, *Proc. Natl. Sci. USA* 88:8972-8976.

          The invention also features recombinant nucleic acid, preferably in a cell or an  
15 organism. The recombinant nucleic acid may contain a sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 or a functional derivative thereof and a vector or a promoter effective to initiate transcription in a host cell. The recombinant nucleic acid can alternatively contain a transcriptional initiation region functional in a cell, a sequence complimentary to an RNA sequence  
20 encoding an hOAT polypeptide and a transcriptional termination region functional in a cell.

          Another aspect of the invention relates to a recombinant cell or tissue comprising a nucleic acid molecule encoding an hOAT polypeptide or hOAT polypeptide fragment. The recombinant cell may comprise a nucleic acid molecule encoding an hOAT  
25 polypeptide; an hOAT domain polypeptide; an hOAT polypeptide fragment or an hOAT polypeptide or polypeptide fragment fused to a non-hOAT polypeptide. The recombinant cell can harbor a nucleic acid vector that is extragenomic or intragenomic.

          Extragenomic vectors are designed with their own origins of replication allowing them to utilize the recombinant cell replication machinery to copy and propagate the  
30 vector nucleic acid sequence. These vectors are small enough that they are not likely to harbor nucleic acid sequences homologous to genomic sequences of the recombinant cell.

          Multiple intragenomic vectors are available to those skilled in the art and contain nucleic acid sequences that are homologous to nucleic acid sequences in a particular

organism's genomic DNA. These homologous sequences will result in recombination events that integrate portions of the vector into the genomic DNA. Those skilled in the art can control which nucleic acid sequences of the vector are integrated into the cell genome by flanking the portion to be incorporated into the genome with homologous sequences in the vector.

A new combination of genes or nucleic acid molecules can be introduced into an organism using a wide array of nucleic acid manipulation techniques available to those skilled in the art.

In yet another aspect, the invention features an isolated, enriched, or purified hOAT polypeptide.

It is also advantageous for some purposes that an amino acid sequence be in purified form. Purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. The substance is preferably free of contamination at a functionally significant level, for example 90%, 95%, or 99% pure. The hOAT polypeptides, or fragments thereof, of the present invention are preferably isolated, purified, or enriched from a mammal or a mammalian cell. The mammal is as defined herein and preferably is a mouse, and most preferably is a human. These polypeptides may be isolated, purified, or enriched from a cell that comprises an endogenous nucleic acid molecule that encodes the polypeptide, or from a cell that is transformed with a nucleic acid molecule that encodes the polypeptide. The polypeptide may also be chemically synthesized. Procedures for obtaining polypeptides using the above methods are well known to those skilled in the art.

The polypeptides of the invention comprise an amino acid sequence having (a) the full-length amino acid sequence encoded by the nucleic acid sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or functional derivatives thereof, or the amino acid sequences set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or functional derivatives thereof.

The invention also features an isolated, enriched, or purified hOAT polypeptide fragment.

Examples of polypeptide fragments include hOAT domains, and hOAT-specific epitopes. Such fragments may, for example, be produced by proteolytic cleavage of the full-length protein. Preferably, the fragment is obtained recombinantly by appropriately

modifying the DNA sequence encoding the proteins to delete one or more amino acids at one or more sites of the C-terminus, N-terminus, and/or within the native sequence.

Fragments of a protein can be used as antigens for the production of hOAT-specific antibodies, or used as competitors of substrates for hOAT polypeptides. It is understood that such fragments may retain one or more characterizing portions of the native complex. Examples of such retained characteristics include: transporting activity; substrate specificity; interaction with other molecules in the intact cell; regulatory functions; or binding with an antibody specific for the native complex, or an epitope thereof.

Well-known examples of domains are the SH2 (Src Homology 2) domain (Sadowski, et al., (1986) *Mol. Cell. Biol.* 6:4396; Pawson and Schlessinger, (1993) *Curr. Biol.* 3:434), the SH3 domain (Mayer, et al., (1988) *Nature* 332:272; Pawson and Schlessinger, (1993) *Curr. Biol.* 3:434), and pleckstrin (PH) domain (Ponting, (1996) *TIBS* 21:245; Haslam, et al., (1993) *Nature* 363:309), all of which are domains that mediate protein:protein interaction, and the kinase catalytic domain (Hanks and Hunter, (1995) *FASEB J* 9:576-595). The relative homology is at least 20%, more preferably at least 30% and most preferably at least 35%. Computer programs designed to detect such homologies are well known in the art.

Comparisons between the sequences of two or more polynucleotides or polypeptides can be performed using the local homology algorithm of Smith and Waterman, (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443, or the method of Pearson and Lipman, (1988) *PNAS* 85:2444. Computer programs implementing these methods can be used and include, BLAST, GAP, BESTFIT, FASTA, and TFASTA which are offered in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.

Protein motifs can be identified using computer programs readily available to those skilled in the art. One such computer program is offered by the Institute for Chemical Research at Kyoto University. The Kyoto computer program is offered at the following web site (<http://www.motif.genome.ad.jp/>).

Protein topology, such as the orientation and location of transmembrane helices can also be identified using readily available computer programs. One such program is TopPred2 offered by Stockholm University and offered at the following web site (<http://www.biokemi.su.se/~server/toppred2/>).

Another aspect of the invention features an isolated, enriched or purified hOAT polypeptide analog.

The hOAT polypeptide analogs of the present invention preferably have a substantially similar biological activity to the proteins encoded by the full-length nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:7, or SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

A sequence that is substantially similar will preferably have at least 85% identity (more preferably at least 90% and most preferably 95-100%) to the amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

An hOAT polypeptide analog will retain some useful function such as, for example, substrate binding, transport activity, or the ability to bind to an hOAT specific antibody (as defined below). The hOAT polypeptide analog may be derived from a naturally occurring complex component by appropriately modifying the protein DNA coding sequence to add, remove, and/or to modify codons for one or more amino acids at one or more sites of the C-terminus, N-terminus, and/or within the native sequence. It is understood that such analogs having added, substituted and/or additional amino acids retain one or more characterizing portions of the native hOAT polypeptides.

Examples of hOAT polypeptides analogs include, but are not limited to, (1) chimeric proteins which comprise a portion of an hOAT polypeptide sequence fused to a non-hOAT polypeptide sequence, for example, a polypeptide sequence of glutathione-S-transferase, (2) hOAT polypeptides lacking a specific domain, for example, the protein kinase C domain, (3) epitope-tagged hOAT polypeptides or fragments for immuno detection or purification and (4) hOAT proteins having a point mutation.

Human OAT polypeptide analogs with deleted, inserted and/or substituted amino acid residues may be prepared using standard techniques well known to those of ordinary skill in the art. For example, the modified components of the analogs may be produced using site-directed mutagenesis techniques (as exemplified by Adelman et al., (1983) *DN4* 2:183 ) wherein nucleotides in the DNA coding the sequence are modified such that a modified coding sequence is produced, and thereafter expressing this recombinant DNA in



a prokaryotic or eukaryotic host cell, using techniques such as those described above. Alternatively, proteins with amino acid deletions, insertions and/or substitutions may be conveniently prepared by direct chemical synthesis, using methods well known in the art. The analogs of the hOAT polypeptides may exhibit the same qualitative biological activity  
5 as the hOAT polypeptides themselves.

In another aspect, the invention features an antibody (e.g., a monoclonal or polyclonal antibody) having specific-binding affinity to an hOAT polypeptide or hOAT polypeptide analog or fragment.

Antibodies having specific-binding affinity to an hOAT polypeptide may be used  
10 in methods for detecting the presence and/or amount of an hOAT polypeptide in a sample by contacting the sample with the antibody under conditions such that an immunocomplex forms and detecting the presence and/or amount of the antibody conjugated to the hOAT polypeptide. Diagnostic kits for performing such methods may be constructed to include a first container containing the antibody and a second container having a conjugate of a  
15 binding partner of the antibody and a label, such as, for example, a radioisotope. The diagnostic kit may also include notification of an FDA approved use and instructions therefor.

For the production of polyclonal antibodies, various host animals may be immunized by injection with the antigen. Various adjuvants may be used to increase the  
20 immunological response, depending on the host species.

Monoclonal antibodies may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. Monoclonal antibodies may be obtained by methods known to those skilled in the art. See, for example, Kohler, et al., (1975) *Nature* 256:495-497, and U.S. Patent No. 4,376,110 issued  
25 March 8, 1983, to David et al.

Still further, the invention features a hybridoma which produces an antibody having specific-binding affinity to an hOAT polypeptide.

In preferred embodiments, the hOAT antibody comprises a sequence of amino acids that is able to specifically bind an hOAT polypeptide.

30 The invention features a method for identifying human cells containing an hOAT polypeptide or a related sequence. The method involves identifying the novel polypeptide in human cells using techniques that are routine and standard in the art, such as those



described herein for identifying hOAT (*e.g.*, cloning, Southern or Northern blot analysis, Western blot analysis, immunoassay, *in situ* hybridization, PCR amplification, *etc.*).

The invention also features methods of screening cells for natural-binding partners of hOAT polypeptides. Binding partners include modulators and downstream signaling molecules such as adaptor proteins and may be identified by techniques well known in the art such as co-immunoprecipitation or by using, for example, a two-hybrid screen. (Fields and Song, U.S. Patent No. 5,283,173, issued February 1, 1994, and incorporated by reference herein). The present invention also features the purified, isolated or enriched versions of the polypeptides identified by the methods described above.

10 The invention provides methods for screening compounds for their ability to inhibit, or modulate the biological activity of the human organic anion transporter molecules of the invention. In preferred embodiments, cells expressing an hOAT polypeptide, including recombinant expression constructs of the invention, are contacted with a compound, and transport activity is assayed. In especially preferred embodiments, competition assays, using known human organic anion transporter substrates, such as *p*-aminohippurate, are used to test the ability of a screened compound to interfere (compete) with the uptake of a known substrate of an hOAT polypeptide. Additional preferred  
15 embodiments comprise quantitative analyses of such effects.

The present invention is also useful for the detection of substrates, analogues of substrates, inhibitors and modulators, heretofore known or unknown, for the transporters of the invention, either naturally occurring or embodied as a drug. In preferred  
20 embodiments, such substrates, analogues, or modulators may be detected in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, vitreous humor, or any other bodily fluid. In additional preferred embodiments, the invention provides methods for detecting and identifying substrates, analogues of substrates, or modulators that preferentially affect  
25 either the uptake function or the efflux function of the transporters of the invention.

One method for identifying a substance capable of modulating an hOAT polypeptide activity comprises the steps of (a) contacting an hOAT polypeptide with a test substance; and (b) determining whether the substance alters the transporting activity of  
30 said polypeptide.

Another method of identifying substances capable of modulating the function of an hOAT polypeptide comprises the following steps: (a) expressing an hOAT polypeptide in

cells; (b) adding a compound to the cells; and (c) monitoring the transporting activity of the hOAT.

A method of identifying substrates or inhibitors of hOAT polypeptides comprises the following steps: (a) adding a test substrate to a cell line expressing an hOAT polypeptide; and (b) measuring the intracellular concentration of the test substrate.

A second method of identifying substrates or inhibitors of hOAT polypeptides comprises the following steps: (a) adding a test substrate to a cell line expressing an hOAT polypeptide; (b) adding a substrate known to be transported by said hOAT polypeptides; (c) measuring whether said test substrate competes with the uptake of the known substrate.

Another method of identifying substrates of an hOAT polypeptide comprises the following steps: (a) attaching a fluorescent compound onto a test compound; (b) adding said compound to a cell line expressing an hOAT polypeptide; and (c) monitoring whether said fluorescent compound is taken into said cell line through the use of fluorescent microscopy or any other method which can detect fluorescence.

In a preferred embodiment, high-throughput screening employing 96-well plates and a microtiter® fluorescence detection system is used to detect potential modulators, substrates, analogues of substrates and inhibitors of hOAT polypeptides which can compete with binding or transport of fluorescent compounds known to bind or be transported by hOAT polypeptides.

In yet another embodiment, uptake of a test substrate by an hOAT expressing cell line is measured using knowledge of an hOAT's antiporter activity. Uptake is measured by monitoring efflux of an accompanying antiporter molecule. Efflux of the antiporter molecule can be detected using techniques known to those skilled in the art, for example, radiolabeling of the effluxed molecule and measurement using a scintillation counter.

Measurement of antiporter efflux is also amenable to high-throughput screening.

A still further aspect of the invention is the identification of substrates, analogues of substrates, inhibitors, or modulators that affect the pharmacokinetics of drugs and compounds transported by hOAT polypeptides. Substances which decrease the transporting activity of hOAT polypeptides are useful for increasing the half-life in the body of drugs and compounds excreted via human organic anion transporters.

Yet, another aspect of the invention involves substrates, and modulators, which increase the transporting activity of an hOAT. Such substances are advantageous for increasing the delivery of a drug or compound, which is transported by an hOAT, to a

target organ such as kidney or liver. For example, a modulator which increases the transporting activity of hOAT1 would increase the delivery of PAH or other drugs or compounds transported by hOAT1 to the kidney.

5 A last aspect of the invention features a cultured cell line which stably expresses an hOAT polypeptide. Such cell lines are useful for identifying substrates which are transported by hOAT polypeptides, inhibitors of hOAT polypeptides and for identifying modulators of hOAT polypeptides. Further, a panel of cells stably expressing hOAT polypeptides will be useful in studying the pharmacokinetics of various drugs and compounds. Preferably, the expression of an hOAT polypeptide in such cell lines is under  
10 the control of an inducible promoter. More preferably, said inducible promoter has a very low basal level of expression in such cell lines and high expression when induced.

A cell line stably expressing an hOAT polypeptide is useful for designing substrates which can be transported by an hOAT polypeptide at either an increased or a decreased rate. Decreasing the rate of transport can increase the effectiveness of some  
15 medicinals by making their clearance from the body occur more slowly.

Cell lines stably expressing hOAT polypeptides are also useful for the identification and development of drugs or compounds targeted to a particular organ. For example, drugs or compounds identified as substrates of hOAT1 would be good candidates for treating diseases of the kidneys whereas those identified as substrates of  
20 hOAT2 would be good candidates for treating diseases of the liver.

The OAT expressing cell lines are also useful for identifying potentially toxic compounds or drugs. For example, newly discovered or modified drugs belonging to a class of drugs known to be toxic to certain organs, e.g., kidney can be tested for transport against the panel of cells expressing human OAT1 or hOAT3 polypeptides. If the new  
25 drug or compound is transported by hOAT1 expressing cells, the new drug or compound may also be toxic to the kidney. However, if the new drug or compound, belonging to a class known to be toxic, is not transported by hOAT1 or hOAT3 expressing cells than the new drug or compound would be expected to be less toxic to the kidney than the parent class of drugs or compounds.

30 A preferred embodiment includes the identification of toxic compounds which are substrates of hOATs, and which can be used to treat diseases of organs or tissues that express hOATs. For example, toxic compounds found to be transported by hOAT1 or hOAT3 would have potential use as anti-cancer drugs to treat cancers of the kidney.

The methods of the present invention can utilize any of the molecules disclosed in the invention. These molecules include nucleic acid molecules encoding hOAT polypeptides, nucleic acid vectors, recombinant cells, polypeptides, or antibodies described herein.

- 5           The summary of the invention described above is non-limiting and other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

### Definitions

- 10           By "isolated" in reference to nucleic acid it is meant a polymer preferably consisting of 14 or more nucleotides conjugated to each other, including DNA or RNA that is isolated from a natural source or that is synthesized. The isolated nucleic acid of the present invention is unique in the sense that it is not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has  
15           been removed from its normal cellular (i.e., chromosomal) environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide sequence present, but that it is essentially free (about 90-95% pure at least) of nucleotide material naturally associated with it and thus is meant to be distinguished from isolated chromosomes.

- 20           By the use of the term "enriched" in reference to nucleic acid it is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2-5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a preferential reduction in the amount of other DNA or RNA present, or by a preferential  
25           increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased.

- The term "significant" here is used to indicate that the level of increase is useful to  
30           the person making such an increase, and generally means an increase relative to other nucleic acids of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no DNA or RNA from other sources. The other source DNA may, for example, comprise DNA from a yeast or bacterial genome, or a

cloning vector such as pUC19. This term distinguishes the sequence from naturally occurring enrichment events, such as viral infection, or tumor type growths, in which the level of one mRNA may be naturally increased relative to other species of mRNA. That is, the term is meant to cover only those situations in which a person has intervened to  
5 elevate the proportion of the desired nucleic acid.

The term "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/mL). The term is also chosen to  
10 distinguish clones already in existence which may encode an hOAT polypeptide but which have not been isolated from other clones in a library of clones. Thus, the term covers clones encoding an hOAT polypeptide which are isolated from other non-hOAT clones.

The term "nucleic acid molecule" describes a polymer of deoxyribonucleotides (DNA) or ribonucleotides (RNA). The nucleic acid molecule may be isolated from a  
15 natural source by cDNA cloning or subtractive hybridization or synthesized manually. The nucleic acid molecule may be synthesized manually by the triester synthetic method or by using an automated DNA synthesizer.

The term "cDNA cloning" refers to hybridizing a small nucleic acid molecule, a probe, to cDNA. The probe hybridizes (binds) to complementary sequences of cDNA.

20 The term "complementary" describes two nucleotides that can form multiple favorable interactions with one another. For example, adenine is complementary to thymine as they can form two hydrogen bonds. Similarly, guanine and cytosine are complementary since they can form three hydrogen bonds. Thus if a nucleic acid sequence contains the following sequence of bases, thymine, adenine, guanine and  
25 cytosine, a "complement" of this nucleic acid molecule would be a molecule containing adenine in the place of thymine, thymine in the place of adenine, cytosine in the place of guanine, and guanine in the place of cytosine. Because the complement can contain a nucleic acid sequence that forms optimal interactions with the parent nucleic acid molecule, such a complement can bind with high affinity to its parent molecule.

30 The term "hybridize" refers to a method of interacting a nucleic acid sequence with a DNA or RNA molecule in solution or on a solid support, such as cellulose or nitrocellulose. If a nucleic acid sequence binds to the DNA or RNA molecule with high affinity, it is said to "hybridize" to the DNA or RNA molecule. The strength of the



interaction between the probing sequence and its target can be assessed by varying the stringency of the hybridization conditions. Under highly stringent hybridization conditions, only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having one or two mismatches out of 20 contiguous nucleotides.

By "conserved nucleic acid regions", it is meant regions present on two or more nucleic acids encoding an hOAT polypeptide, to which a particular nucleic acid sequence can hybridize under lower stringency conditions. Examples of lower stringency conditions suitable for screening for nucleic acids encoding hOAT polypeptides are provided in Abe, et al. (1992) *J. Biol. Chem.* 19:13361 (hereby incorporated by reference herein in its entirety, including any drawings). Preferably, conserved regions differ by no more than 5 out of 20 contiguous nucleotides.

By "unique nucleic acid region" is meant a sequence present in a full-length nucleic acid coding for an hOAT polypeptide that is not present in a sequence coding for any other known naturally occurring polypeptide. Such regions preferably comprise 12 or more contiguous nucleotides present in the full-length nucleic acid encoding an hOAT polypeptide. In particular, a unique nucleic acid region is preferably of human origin.

The term "nucleic acid vector" relates to a single or double stranded circular nucleic acid molecule that can be transfected or transformed into cells and replicate independently or within the host cell genome.

The terms "transformation" and "transfection" refer to methods of inserting an expression construct into a cellular organism.

The term "promoter element" describes a nucleotide sequence that is incorporated into a vector that, once inside an appropriate cell, can facilitate transcription factor and/or polymerase binding and subsequent transcription of portions of the vector DNA into mRNA. The promoter element precedes the 5' end of the hOAT polypeptide nucleic acid molecule such that the latter is transcribed into mRNA. Host cell machinery then translates mRNA into a polypeptide.

By an "hOAT polypeptide" is meant the full-length amino acid sequence of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof.

The term "recombinant cell" refers to a cell that has been altered to contain a new combination of genes or nucleic acid molecules.



The term "recombinant organism" refers to an organism that has a new combination of genes or nucleic acid molecules.

The term "organism" relates to any living being comprised of at least one cell. An organism can be as simple as one eukaryotic cell or as complex as a mammal. Therefore, a recombinant organism can also be a recombinant cell, which may be a eukaryotic or a prokaryotic organism.

By "recombinant hOAT polypeptide" it is meant to include a polypeptide produced by recombinant DNA techniques such that it is distinct from a naturally occurring polypeptide either in its location (*e.g.*, present in a different cell or tissue than found in nature), purity or structure. Generally, such a recombinant polypeptide will be present in a cell in an amount different from that normally observed in nature.

The term "eukaryote" refers to an organism comprised of cells that contain a nucleus. Eukaryotes are differentiated from "prokaryotes" which do not have a nucleus and lack other cellular structures found in eukaryotes, such as mitochondria and endoplasmic reticulum. Prokaryotes include unicellular organisms, such as bacteria, while eukaryotes are represented by yeast, invertebrates, and vertebrates.

The term "extragenomic" refers to a nucleic acid vector which does not insert into the cell genome. Thus, these vectors replicate independently of the host genome and do not recombine with or integrate into the genome.

The term "intragenomic" defines a nucleic acid construct that is incorporated within the cell genome.

By "isolated" in reference to a polypeptide is meant a polymer of 6, 12, 18 or more amino acids conjugated to each other, including polypeptides that are isolated from a natural source or that are synthesized. The isolated polypeptides of the present invention are unique in the sense that they are not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only amino acid chain present, but that it is essentially free (about 90-95% pure at least) of material naturally associated with it.

By the use of the term "enriched" in reference to a polypeptide it is meant that the specific amino acid sequence constitutes a significantly higher fraction (2-5 fold) of the total of amino acid sequences present in the cells or solution of interest than in normal or

diseased cells or in the cells from which the sequence was taken. This could be caused by a preferential reduction in the amount of other amino acid sequences present, or by a preferential increase in the amount of the specific amino acid sequence of interest, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other amino acid sequences present, just that the relative amount of the sequence of interest has been significantly increased.

The term "significant" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other amino acid sequences of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there are no amino acid sequences from other sources. The other source amino acid sequences may, for example, comprise amino acid sequences encoded by a yeast or bacterial genome, or a cloning vector such as pUC19. The term is meant to cover only those situations in which a person has intervened to elevate the proportion of the desired amino acid sequences.

The term "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/mL).

By "an hOAT polypeptide fragment" it is meant an amino acid sequence that is less than the full-length hOAT amino acid sequences (and which excludes the listed EST sequences) encoded by the nucleic acid sequence set forth in SEQ ID NO:1 (excluding EST clone R25797), SEQ ID NO:2 (excluding EST clone A1016020), SEQ ID NO:3 (excluding EST clone A 1016020), SEQ ID NO:4 (excluding EST clone H41333), SEQ ID NO:5, or SEQ ID NO: 6 (excluding EST clone AA705512) or the amino acid sequence (excluding the amino acids encoded by the EST clones previously listed set forth in SEQ ID NO:7, SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12. Thus, the term "fragment" is used to indicate a polypeptide derived from the amino acid sequence of the hOAT polypeptides, of the complexes having a length less than the full-length polypeptide from which it has been derived.

By "an hOAT domain" it is meant a portion of the hOAT polypeptide having homology to amino acid sequences from one or more known proteins wherein the sequence predicts some common function, interaction or activity.

By "hOAT polypeptide analog" it is meant an amino acid sequence substantially similar to the sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:7, or SEQ ID NO:8; SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

By "biological activity" it is meant an activity of the hOAT polypeptides in a cell. The biological activity of the hOAT polypeptides is related to some of the activities of the cell which include, but are not limited to, transport (uptake or excretion of a substrate from a cell), cell proliferation, mitogenesis, metastasis, tumor escape, cell adhesion, transformation, or apoptosis.

By "identity" is meant a property of sequences that measures their similarity or relationship. Identity is measured by dividing the number of identical residues in the two sequences by the total number of residues and multiplying the product by 100. Thus, two copies of exactly the same sequence have 100% identity, but sequences that are less highly conserved and have deletions, additions, or replacements have a lower degree of identity. Those skilled in the art will recognize that several computer programs are available for determining sequence identity.

An hOAT polypeptide analog may differ from the native sequence of an hOAT polypeptide in that one or more amino acids have been changed, added or deleted. Changes in amino acids may be conservative or non-conservative. By "conservative" it is meant the substitution of an amino acid for one with similar properties such as charge, hydrophobicity, structure, etc.

By "hOAT-specific epitope" it is meant a sequence of amino acids that is both antigenic and unique to hOAT.

By "specific-binding affinity" is meant that the antibody binds to target (hOAT) polypeptides with greater affinity than it binds to other polypeptides under specified conditions.

The term "polyclonal" refers to antibodies that are heterogenous populations of antibody molecules derived from the sera of animals immunized with an antigen or an antigenic functional derivative thereof.

"Monoclonal antibodies" are substantially homogenous populations of antibodies to a particular antigen.

The term "antibody fragment" refers to a portion of an antibody, often the hypervariable region and portions of the surrounding heavy and light chains, that displays specific binding affinity for a particular molecule. A hypervariable region is a portion of an antibody that physically binds to the polypeptide target.

5 By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example an hOAT antibody.

By "natural-binding partner" it is meant a protein, organic anion, or other molecule that interacts with an hOAT polypeptide.

10 As used herein, pharmacokinetics is the process by which a drug or compound is absorbed, distributed, metabolized and eliminated by the body.

The term "compound" includes small organic or inorganic molecules of molecular weight of preferably less than 1000 atomic units, more preferably less than 800 atomic units, and most preferably less than 500 atomic units. "Organic molecules" include all molecules that contain a carbon atom, whereas "inorganic molecules" are those that do not  
15 have a carbon atom.

The term "function" refers to the cellular role of a protein. The role of the proteins of the invention may include transport of substrates into and out of a cell, involvement in cascades controlling cell growth, migration, differentiation, gene expression, muscle contraction, glucose metabolism, cellular protein synthesis, and regulation of the cell  
20 cycle.

The term "modulates" refers to the ability of a compound to alter the function of an hOAT polypeptide. A modulator preferably activates or decreases the transporter activity of an hOAT protein. A modulator that increases the transporting activity is a positive modulator; and one that decreases the transporting activity is a negative modulator. The  
25 term "modulates" also refers to altering the function of a protein by increasing or decreasing the probability that a complex, i.e. an assembly of at least two molecules bound to one another, forms between an hOAT protein and a natural-binding partner.

The term "transporting activity," in the context of the invention, defines the ability of a transporter polypeptide to uptake a substrate into a cell or efflux a molecule out of a  
30 cell.

The term "substrate" as used herein refers to a molecule that is transported into or out of a cell by an OAT polypeptide. The substrate may be an organic compound or molecule, inorganic compound or molecule, a peptide, or a protein.

The term "activates" refers to increasing the transport or efflux of a molecule into or out of a cell.

The term "inhibitor" refers to a compound or substance that binds to a substrate-binding site and, thereby, decreases or prevents transport of an hOAT substrate.

5 The term "expressing" as used herein refers to the production of an hOAT polypeptide from a nucleic acid vector containing an hOAT gene within a cell.

The term "adding" as used herein refers to administering a solution comprising a compound to the medium bathing cells.

10 The term "functional derivative" with respect to a polypeptide is a polypeptide that possesses a biological activity (either functional or structural) or an immunological characteristic that is substantially similar to a biological activity or an immunological characteristic of a non-recombinant hOAT. A functional derivative of an hOAT polypeptide may or may not contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance  
15 of a specific function. The term "functional derivative" is intended to include the "fragments", "variants", "analogues", "homologues" or chemical derivatives of a molecule.

Similarly, a "functional derivative" of a gene encoding an hOAT polypeptide of the present invention includes "fragments", "variants", or "analogues" of the gene, which  
20 may be "substantially similar" in nucleotide sequence, and which encode a molecule possessing similar activity to an hOAT polypeptide or fragment thereof. Permutations resulting from degeneracy of the genetic code are also considered functional derivatives.

A molecule is said to be "substantially similar" to another molecule if the sequence of amino acids in both molecules is substantially the same. Substantially similar amino  
25 acid molecules will possess a similar biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if one of the molecules contains additional amino acid residues not found in the other, or if the sequence of amino acid residues is not identical.

A "chemical derivative" of a polypeptide contains additional chemical moieties not  
30 normally a part of the polypeptide.

The term "mammalian" refers to such organisms as mice, rats, rabbits, goats, more preferably monkeys and apes, and most preferably humans.

**Brief Description of the Figures**

FIG. 1 shows the cDNA and deduced amino acid sequences of human OAT1.

FIG. 2 shows the cDNA and deduced amino acid sequences of human OAT2A.

FIG. 3 shows the cDNA and deduced amino acid sequences of human OAT2B.

5 FIG. 4 shows the cDNA and deduced amino acid sequences of human OAT3.

FIG. 5 shows the cDNA and deduced amino acid sequences of human OAT4.

FIG. 6 shows the cDNA and deduced amino acid sequences of human OAT5.

FIG. 7 shows the amount of  $^3\text{H}$ -PAH taken up by HeLa cells transfected with increasing amounts of hOAT1 or vector control (LacZ) cDNAs.

10 FIG. 8 shows that oocytes injected with hOAT1 RNA uptake  $^3\text{H}$ -PAH to a much greater extent than water injected oocytes.

FIG. 9 shows that ECR293 cells stably containing hOAT1, in plasmid pIND(sp1), uptake approximately 50-fold more  $^3\text{H}$ -PAH when induced by ponasterone than non-induced cells.

15 FIG. 10 shows that ECR293 cells stably containing hOAT2A, in plasmid pIND(sp1), uptake approximately 2.6-fold more  $^3\text{H}$ -PAH when induced by ponasterone than non-induced cells.

FIG. 11 shows that sulfobromophthalein but not  $\alpha$ -ketoglutarate competed with  $^3\text{H}$ -PAH uptake in ECR293/OAT2A cells induced with ponasterone.

20 FIG. 12 shows that oocytes injected with hOAT3 RNA uptake  $^3\text{H}$ -PAH to a much greater extent than water injected oocytes.

FIG. 13 (A-E) shows Northern Blot analyses of hOAT1-5 mRNA in various human tissues.

25

**DETAILED DESCRIPTION OF THE INVENTION**

30 A. **Introduction**

Organic anions are carbon-based molecules with one or more negative charges at physiological pH. They can be endogenous compounds such as bile acids or xenobiotics such as *p*-aminohippurate. Organic anion transporters are membrane proteins that



facilitate the transport of organic anions across the cell membrane. The family described herein consists of proteins sharing at least 50% amino acid sequence similarity among each other. They show broad substrate specificity, meaning that each member can transport a variety of organic anions. Hence, they are often referred to as multi-specific organic anion transporters. The mechanism of transport, though not currently elucidated, probably is a secondary or tertiary active transport involving exchange of another organic anion. An example of a tertiary transport system is the rat kidney OAT1 transporter. The primary transport system is the  $\text{Na}^+/\text{K}^+$ -ATPase, which establishes the high out-to-in  $\text{Na}^+$  gradient. This  $\text{Na}^+$  gradient then drives the cotransport of  $\text{Na}^+$  and dicarboxylates such as  $\alpha$ -ketoglutarate through a secondary  $\text{Na}^+$ /dicarboxylate transporter. Finally, the tertiary transport system is established by OAT1, which exchanges external PAH for intracellular  $\alpha$ -ketoglutarate.

The liver and kidney are two organs rich in organic anion transporters. Hepatocytes and epithelial cells of the kidney proximal tubules are polarized cells with a basolateral and apical membrane. For the hepatocyte, the basolateral membrane faces the blood-filled sinusoid. Thus, the basolateral membrane is also called the sinusoidal membrane. The apical membrane faces the canaliculus, into which bile is secreted. The hepatocyte thus can transport organic anions from the blood into the bile canaliculus across the basolateral and apical membranes. Similarly, cells of the kidney proximal tubule transport organic anions from the blood into urine across their basolateral and apical membranes. Examples of liver sinusoidal transporters are the oatp1 and oatp2, whereas OAT1 is a kidney basolateral transporter.

## **B. General Method**

The practice of the present invention employs conventional techniques of molecular and cell biology, microbiology, and recombinant DNA. Most techniques are well-described in J. Sambrook et al., "Molecular Cloning; A Laboratory Manual" (1989) and J. Celis, ed. "Cell Biology: A Laboratory Handbook" (1998).

## **30 Identification of hOAT Nucleic Acid Molecules**

Extensive searches of Genbank databases were performed using database tools available through the website maintained by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). In particular, the Basic Local Alignment

Search Tool (BLAST) was used to perform sequence similarity searches of the Genbank databases, such as the database containing human expressed sequence tags (ESTs). The EST database contains sequences of randomly selected clones from a variety of human cDNA libraries. Since each EST clone was sequenced with one primer, about 300-500 bp  
5 of sequence information is available from each clone. The BLAST programs can translate these sequences in all possible reading frames and compare them to an input protein sequence. Human ESTs showing homology to rat OAT proteins were hereby identified.

Identified EST clones can be obtained from licensed distributors of the I.M.A.G.E. Consortium Lawrence Livermore National Laboratory (LLNL) cDNA clones. The clones  
10 are then sequenced to verify their homologies to published sequences. DNA sequencing is usually done by Sanger's method of using dideoxynucleotide triphosphates in a primer extension reaction. The DNA to be sequenced, called the template, is first denatured and then annealed to a specific primer, usually between 15-25 basepairs. In one method, <sup>35</sup>S-adenosine triphosphate is incorporated into the growing DNA strand by a DNA  
15 polymerase. Materials for such a reaction are available from commercial kits such as Sequenase 2.0<sup>®</sup> from Amersham. Reaction products are separated on a long polyacrylamide gel, usually 6.0%, by electrophoresis using Tris-borate buffer. After electrophoresis, the gel is dried under vacuum and then exposed to autoradiographic film. The DNA sequence can be read from the autorad image and entered into computer for  
20 sequence analysis. Alternatively, fluorescent-tagged nucleotides can be incorporated by the polymerase chain reaction (PCR) into the template DNA using the thermal-stable polymerase from *Thermus aquaticus* (*Taq* polymerase). The PCR products can then be loaded onto an automated sequencer such as the ABI 377<sup>®</sup> (PE Applied Biosystems, Foster City, CA), which separates the products and determines the sequence.

25 Isolation of cDNA clones from a particular tissue requires the construction of a cDNA library representing messages of that tissue. Library construction entails the isolation of mRNA from the source tissue and usually a fractionation step to separate low (< 0.5 kb) and high molecular weight species. The mRNAs are then primed with a DNA oligonucleotide, either a poly-thymidine or a mixture of random primers. Reverse  
30 transcriptase is then added to synthesize the complementary DNA strand. Short DNA fragments with sticky ends, called adapters, are ligated to the cDNAs so they can be subcloned into an appropriate vector. Popular vectors include bacteriophages such as  $\lambda$ gt11 or  $\lambda$ ZAP (Stratagene, La Jolla, CA). For phage libraries, the cDNAs are ligated to

lambda phage arms at the adapter site and then packaged into phage particles. Once packaged, the library can then be amplified by infecting an appropriate bacterial host. Ready-made libraries of various human tissues are available from several commercial sources.

- 5 To isolate a particular gene of interest from the cDNA library, an aliquot is plated out onto large plates and then transferred to nitrocellulose or nylon membrane. The phage DNA attached to the membrane is denatured in alkali solution, neutralized, and then crosslinked to the filter by baking or UV irradiation. The filter is then hybridized with a probe of interest. The probe can be an oligonucleotide or a longer, double-stranded
- 10 fragment of DNA, such as the insert of an EST clone. The double-stranded probe is usually labeled with  $^{32}\text{P}$  to high specific activity ( $>10^9$  dpm/ $\mu\text{g}$ ), denatured by boiling for 2 minutes, and added to a hybridization buffer to concentration of  $10^6$  dpm/ml. Hybridization buffers can vary in composition depending on the hybridization condition desired. One common formulation contains 0.9 M NaCl, 90 mM sodium citrate (pH 7.0),
- 15 10 mM EDTA (pH 8.0), 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinylpyrrolidone, 0.1 (w/v) BSA, 0.5 % SDS, and 100  $\mu\text{g/ml}$  sheared, denatured salmon sperm DNA. Other formulations are available from commercial sources. The filters are hybridized with the probe at  $65^\circ\text{C}$  overnight or at least 16 hours. They are then washed with sodium citrate solutions with decreasing ionic strength and increasing temperature. Finally, they are
- 20 exposed to autoradiographic film with an intensifying screen at  $-80^\circ\text{C}$  overnight.

- If a plaque contains DNA sequences complementary to the probe, the probe will hybridize to the complementary sequences on the filter. The plaque containing the complementary sequences is detected by exposing X-ray film to the filter. The plaque of interest can then be isolated and purified by conventional methods. Furthermore, once
- 25 purified, the phage DNA can be manipulated so the relevant cDNA insert is excised from the phage into a phagemid by an *in vivo* excision procedure using appropriate host cells. The phagemids can then be amplified in bacteria and isolated by conventional methods such as alkaline lysis. Once the plasmid is purified, it can be analyzed by standard means such as restriction endonuclease or PCR mapping and DNA sequencing. DNA sequence
- 30 information is analyzed for open reading frames and compared to published sequences. As discussed earlier, sets of computer programs for DNA and protein sequence analysis are commercially available.

Once a cDNA coding for a full-length transporter protein is isolated, it can be excised from its cloning vector and subcloned into an appropriate expression vector. Subcloning is a frequently used procedure in recombinant DNA technology. It involves the digestion of DNA by restriction endonucleases, fractionation of the DNA fragments by agarose gel electrophoresis, and purification of the desired fragment by a DNA-binding matrix. The purified fragment is then ligated to a precut vector of choice by adding an enzyme such as T4 DNA ligase. The new DNA construct can then be transfected into a mammalian cell line to test for functional expression. Many types of expression plasmids are commercially available and they all share common elements. An expression plasmid usually contains a bacterial origin of replication, a DNA promoter that is active in a mammalian cell line, a multiple cloning site, a transcriptional termination element, and a selective marker that confers resistance to an antibiotic. An expression plasmid harboring the cDNA of interest can be transfected into mammalian cells by several conventional means like calcium phosphate precipitation or lipofection. Many transfection kits are commercially available.

#### **Recombinant Cells Containing a Nucleic Acid Molecule Encoding an hOAT**

Cell lines which stably express hOAT polypeptides can be isolated. Such cells may be obtained by using expression vectors which carry nucleic acid sequences which encode hOAT polypeptides. The expression vectors may contain viral origins of replication and/or endogenous expression elements. Further, such expression vectors may carry nucleic acid sequences which encode for a selectable marker.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of

transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence encoding an hOAT polypeptide of the invention may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding an hOAT polypeptide of the invention, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

Two DNA sequences (such as a promoter region sequence and a sequence encoding an hOAT polypeptide of the invention) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of a gene sequence encoding an hOAT polypeptide of the invention, or (3) interfere with the ability of the gene sequence of an hOAT polypeptide of the invention to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. Thus, to express a gene encoding an hOAT polypeptide of the invention, transcriptional and translational signals recognized by an appropriate host are necessary.

The present invention encompasses the expression of a gene encoding an hOAT polypeptide of the invention (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, very efficient and convenient for the production of recombinant proteins and for the propagation of clones. Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, including other bacterial strains.

In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors may include  $\lambda$ gt10,  $\lambda$ gt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.



Recognized prokaryotic hosts include bacteria such as *E. coli*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Salmonella*, *Serratia*, and the like. However, under such conditions, the polypeptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

5 To express an hOAT polypeptide of the invention (or a functional derivative thereof) in a prokaryotic cell, it is necessary to operably link the sequence encoding the hOAT polypeptide of the invention to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (*i.e.*, inducible or derepressible). Examples of constitutive promoters include the *int* promoter of  
10 bacteriophage  $\lambda$ , the *bla* promoter of the  $\beta$ -lactamase gene sequence of pBR322, and the *cat* promoter of the chloramphenicol acetyl transferase gene sequence of pPR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage  $\lambda$  ( $P_L$  and  $P_R$ ), the *trp*, *recA*, *lacZ*, *lacI*, and *gal* promoters of *E. coli*, the  $\alpha$ -amylase (Ulmanen *et al.*, (1985) *J. Bacteriol.* 162:176-182), and the  
15 promoters of the bacteriophages of *Bacillus* (Gryczan, In: The Molecular Biology of the Bacilli, Academic Press, Inc., NY, 1982), and *Streptomyces* promoters (Ward *et al.*, (1986) *Mol. Gen. Genet.* 203:468-478). Prokaryotic promoters are reviewed by Glick (1987, *Ind. Microbiol.* 1:277-282), Cenatiempo (1986, *Biochimie* 68:505-516), and Gottesman (1984, *Ann. Rev. Genet.* 18:415-442).

20 Proper expression in a prokaryotic cell also requires the presence of a ribosome-binding site upstream of the gene sequence-encoding sequence. Such ribosome-binding sites are disclosed, for example, by Gold *et al.* (1981, *Ann. Rev. Microbiol.* 35:365-404). The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein,  
25 "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant  
30 progeny have the same functionality as that of the originally transformed cell.

Host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the hOAT polypeptide of interest. Especially suitable hosts for expressing the polypeptides of



the invention are eukaryotic cells. Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, amphibian oocytes, mammalian cells either *in vivo*, or in tissue culture. Mammalian cells which may be useful as hosts include 3T3-L1 preadipocytes, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives. Preferred mammalian host cells include HeLa, EcR293, SP2/0 and J558L, as well as neuroblastoma cell lines such as IMR 332, which may provide better capacities for correct post-translational processing.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences. Another preferred host is an insect cell, for example the *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used (Rubin, (1988) *Science* 240:1453-1459). Alternatively, baculovirus vectors can be engineered to express large amounts of hOAT polypeptides of the invention in insect cells (Jasny, (1987) *Science* 238:1653; Miller *et al.*, In: Genetic Engineering, Vol. 8, Plenum, Setlow *et al.*, eds., pp. 277-297, 1986).

Any of a series of yeast expression systems can be utilized which incorporate promoter and termination elements from the actively expressed sequences coding for glycolytic enzymes that are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals. Yeast provides substantial advantages in that it can also carry out post-translational modifications. A number of recombinant DNA strategies exist utilizing strong promoter sequences and high copy number plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian genes and secretes peptides bearing leader sequences (*i.e.*, pre-peptides). Several possible vector systems are available for the expression of hOAT polypeptides of the invention in a mammalian host.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, cytomegalovirus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen,

myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or  
5 are subject to chemical (such as metabolite) regulation.

Expression of hOAT polypeptides of the invention in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter from the immediate-early gene of human  
10 cytomegalovirus (CMV) (Boshart M. et al., (1985) *Cell* 2:521-30, the promoter of the mouse metallothionein I gene sequence (Hamer et al., (1982) *J. Mol. Appl. Gen.* 1:273-288); the TK promoter of Herpes virus (McKnight, (1982) *Cell* 31:355-365); the SV40 early promoter (Benoist et al., (1981) *Nature* (London) 290:304-31); and the yeast gal4 gene sequence promoter (Johnston et al., (1982) *Proc. Natl. Acad. Sci. (USA)* 79:6971-  
15 6975; Silver et al., (1984) *Proc. Natl. Acad. Sci. (USA)* 81:5951-5955).

Translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes an hOAT polypeptide of the invention (or a functional derivative thereof) does not contain any intervening codons  
20 which are capable of encoding a methionine (*i.e.*, AUG). The presence of such codons results either in the formation of a fusion protein (if the AUG codon is in the same reading frame as the hOAT polypeptide of the invention coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the hOAT polypeptide of the invention coding sequence).

25 A nucleic acid molecule encoding an hOAT polypeptide of the invention and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a nonreplicating DNA or RNA molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the  
30 transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

A vector may be employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the

introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama (1983, *Mol. Cell. Biol.* 3:280-289).

The introduced nucleic acid molecule can be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184; "Molecular Cloning: A Laboratory Manual", 1989, supra). *Bacillus* plasmids include pC194, pC221, pT127, and the like (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, NY, pp. 307-329, 1982). Suitable *Streptomyces* plasmids include p1J101 (Kendall *et al.*, (1987) *J. Bacteriol.* 169:4177-4183), *Pseudomonas* plasmids are reviewed in John *et al.* (1986) *Rev. Infect. Dis.* 8:693-704), and Izaki (1978) *J. Bacteriol.* 33:729-742).

Preferred eukaryotic plasmids include, for example, pcDNA3.1 (Invitrogen), BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein *et al.*, (1982) Miami Wntr. Symp. 19:265-274; Broach, In: "The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470, 1981; Broach, (1982) *Cell* 28:203-204; Bollon *et al.*, (1980) *J. Clin. Hematol. Oncol.* 10:39-48; Maniatis, In: *Cell Biology: A Comprehensive Treatise*, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608, 1980).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, *i.e.*, transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene(s) results in the production of an hOAT polypeptide of the invention, or fragments thereof. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like). A variety of incubation conditions can be used to form the peptide of the present invention. The most preferred conditions are those which mimic physiological conditions.

Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture technique appropriate to the cell type.

Cell lines stably expressing human organic anion transporters under the control of an inducible promoter can also be isolated. For inducible expression of human organic anion transporters, the ecdysone system from Invitrogen (San Diego, CA) was used. Ecdysone is a hormone that regulates metamorphosis in insects, such as the fruitfly *Drosophila*. Like other steroid hormones, ecdysone exerts its effects by binding to a nuclear receptor, the VgR. The hormone-receptor complex then binds to a sequence of DNA called the ecdysone response element (EcRE), and activates transcription from promoters containing these elements. Since mammalian cells do not express ecdysone receptors, they normally do not respond to the insect hormone. However, if a piece of DNA under the control of an ecdysone inducible promoter is transfected into a mammalian cell line, treating the cell line with ecdysone will induce expression of the said DNA.

The cloned transporters can also be expressed in the amphibian oocytes, a widely used heterologous system for the expression of membrane proteins. Complementary RNA can be synthesized from purified cDNA *in vitro* if it is downstream of an appropriate promoter, such as the T3 or T7 phage promoter. The synthesized RNA are microinjected

into each frog oocyte, which translates the message and expresses the protein in its membranes. *Xenopus* oocytes can be obtained by surgical removal of ovaries from the frog *Xenopus laevis*. The ovary lobes are treated with collagenase to remove the vitellin layer. Oocytes can be maintained for up to two weeks in Barth solution ( 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO<sub>3</sub>, 15 mM HEPES (pH 7.6), 0.7 mM CaCl<sub>2</sub>, 0.82 mM MgSO<sub>4</sub> and 50 µg/ml gentamicin) at 16°C. Typically, about 50 nl or 10-30 ng of RNA are injected into each oocyte. The injected oocytes are maintained at 16°C for 2-3 days and then subjected to transport assays.

#### Assays for functional expression

Assaying for functional expression of transporters can be done using a labeled compound that is a known substrate of the transporter. For hOAT1, *p*-aminohippurate (PAH) is a high affinity substrate that is rapidly transported. Transfected cells or injected oocytes are washed briefly with media or Barth buffer and then incubated with the labeled PAH. After the desired incubation time, the excess label is washed away by adding cold buffer. The cells are then solubilized with SDS and the amount of label taken up can be determined by scintillation counting.

Other methods for measuring transport activity of organic anion transporters are well known in the art and are applicable to the present invention. For example, transport assays utilizing membrane vesicles was demonstrated by Yamazaki, et al., (1997) *Drug Metabolism and Disposition*, 1123-1129; and Pascolo, et al. (1998) *Biochem J.* 331:99-103.

#### Cell lines stably expressing hOAT polypeptides used to identify substrates, inhibitors and modulators of hOAT polypeptides

Cell lines stably expressing hOAT polypeptides can be used to screen for other substrates or inhibitors of hOAT polypeptides through use of a competition assay. If a test compound is well transported by an hOAT protein, it will compete with a substrate, known to be transported by a particular hOAT, for access to the transporter. Cells expressing hOAT proteins would then uptake less of the known substrate in the presence than in the absence of the competitor.

If a test compound competes with transport of a known substrate, further testing is necessary to confirm that the test compound is a substrate or inhibitor. Such confirmation can be done using techniques known to those skilled in the art, for example, the intracellular concentration of the test compound can be measured using HPLC. If a test



compound, which competes with transport of a known substrate, is also found to accumulate inside a cell, this confirms that the test substrate is a substrate and not an inhibitor of the particular hOAT being tested.

Modulators can be identified by adding a test compound to cell lines stably expressing hOAT polypeptides and measuring whether the addition of the test compound increases or decreases the transport of hOAT substrates. If a modulator is identified, which decreases or inhibits the transport of a substrate, further testing, using techniques known to those skilled in the art, is necessary to confirm that such modulators are not actually competitive inhibitors. One such technique involves adding more substrate in a competition assay to determine if said substrate can compete with the inhibitory effects of the putative modulator. If adding more substrate has no effect on the modulating activity then the modulator is not a competitive inhibitor.

#### Nucleic Acid Probes, Methods, and Kits for Detection of the hOAT Polypeptides

A nucleic acid probe of the present invention may be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain other nucleic acid molecules of the present invention. A chromosomal DNA or cDNA library may be prepared from appropriate cells according to recognized methods in the art (cf. "Molecular Cloning: A Laboratory Manual", second edition, Cold Spring Harbor Laboratory, Sambrook, Fritsch, & Maniatis, eds., 1989).

In the alternative, chemical synthesis can be carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the polypeptide of interest. The synthesized nucleic acid probes may be used as primers in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques, essentially according to PCR Protocols, "A Guide to Methods and Applications", Academic Press, Michael, *et al.*, eds., 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art ("Molecular Cloning: A Laboratory Manual", 1989, *supra*). The hybridization probes of the present invention can be labeled by standard labeling techniques such as



with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes may be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art. The nucleic acid probe may be immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The samples used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample that is compatible with the method utilized.

One method of detecting the presence of nucleic acids of the invention in a sample comprises (a) contacting the sample with the above-described nucleic acid probe under conditions such that hybridization occurs, and (b) detecting the presence of the probe bound to the nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

A kit for detecting the presence of nucleic acids of the invention in a sample comprises at least one container means having disposed therein the above-described nucleic acid probe. The kit may further comprise other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabeled probes, enzymatic labeled probes (horseradish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin). Preferably, the kit further comprises instructions for use.

### Antibodies, Hybridomas, Methods of Use and Kits for Detection of the hOAT Polypeptides

The present invention relates to an antibody having binding affinity to an hOAT polypeptide of the invention. The polypeptide may have the amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a functional derivative thereof, or the amino acid sequence set forth in SEQ ID NO:7 or SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or a functional derivative thereof, or at least 9 contiguous amino acids thereof (preferably, at least 20, 30, 40, 50 or 60 contiguous amino acids thereof).

The present invention also relates to an antibody having specific binding affinity to an hOAT polypeptide of the invention. Such an antibody may be isolated by comparing its binding affinity to an hOAT polypeptide of the invention with its binding affinity to other polypeptides. Those which bind selectively to an hOAT polypeptide of the invention would be chosen for use in methods requiring a distinction between an hOAT polypeptide of the invention and other polypeptides, including other polypeptides with similar amino acid sequences. Such methods could include, but should not be limited to, the analysis of altered hOAT polypeptide expression in tissue containing other polypeptides.

The hOAT polypeptides of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide could be generated as described herein and used as an immunogen. The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the present invention may be generated using one of the procedures known in the art such as chimerization or CDR grafting.

The present invention also relates to hybridomas that produce the above-described monoclonal antibodies, or binding fragment thereof. A hybridoma is an immortalized cell line that is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands, 1984; St. Groth *et al.*, (1980), *J. Immunol. Methods* 35:1-21). Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well

known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal that is immunized, the antigenicity of the polypeptide and the site of injection.

5           The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or  $\beta$ -galactosidase) or through the inclusion of an adjuvant during immunization.

10           For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Agl4 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell that produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay,  
15   western blot analysis, or radioimmunoassay (Lutz *et al.*, (1988) *Exp. Cell Res.* 175:109-124). Hybridomas secreting the desired antibodies are cloned and the class and subclass are determined using procedures known in the art (Campbell, (1984) "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", *supra*).

20           For polyclonal antibodies, antibody-containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The above-described antibodies may be detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such  
25   as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, *see* Stemberger *et al.*, (1970) *J. Histochem. Cytochem.* 18:315; Bayer *et al.*, (1979) *Meth. Enzym.* 62:308; Engval *et al.*, (1972) *Immunol.* 109:129; Goding, J. (1976) *Immunol. Meth.* 13:215). The labeled  
30   antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues that express a specific peptide.

          The above-described antibodies may also be immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex

carbohydrates such as agarose and sepharose, acrylic resins such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10, 1986; Jacoby *et al.*, (1974) Meth. Enzym. 34, Academic Press, N.Y.). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as in immunochromatography.

The present invention also encompasses a method of detecting an hOAT polypeptide in a sample, comprising: (a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and (b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard ("An Introduction to Radioimmunoassay and Related Techniques" Elsevier Science Publishers, Amsterdam, The Netherlands, 1986), Bullock *et al.* ("Techniques in Immunocytochemistry," Academic Press, Orlando, FL Vol. 1, 1982; Vol. 2, 1983; Vol. 3, 1985), Tijssen ("Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands, 1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test samples used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can readily be adapted in order to obtain a sample which is testable with the system utilized.

A kit contains all the necessary reagents to carry out the previously described methods of detection. The kit may comprise: (i) a first container means containing an

above-described antibody, and (ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. Preferably, the kit also contains instructions for use. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and  
5 reagents capable of detecting the presence of bound antibodies.

Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents that are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe  
10 kits. One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats that are well known in the art.

### Examples

Example 1: Identification of human EST clones homologous to rat organic  
15 anion transporter proteins

The cloning of rat kidney organic anion transporter OAT1 (Sekine, *et al.*, (1997) *J. Biol. Chem.* 30: 18526-18529) and liver transporter OAT2 (Sekine, *et al.*, (1998) *FEBS Letters* 429: 179-182) has been previously reported. The published amino acid sequences of OAT1 and OAT2 from rat were used as input sequences to perform a BLAST search of  
20 human expressed sequence tags (EST) sequences deposited in Genbank. The BLAST search identified several sequences that show amino acid homology to rat OAT1 and OAT2.

EST clones accession Nos. R25797 and A1016020 show high homology to rat OAT1 and OAT2, respectively. The clones isolated using EST clones R25797 and  
25 A1016020 were named hOAT1 and hOAT2, respectively. The other ESTs show lower homology to rat OAT1 and OAT2. These other EST clones have been named hOAT3, hOAT4, and hOAT5. The latter three clones are not considered human homologues to the rat OAT1 and OAT2.



Example 2: Isolation of full-length human OAT1 cDNA

The human EST clone R25797, listed in Example 1, was purchased from a distributor of IMAGE Consortium (LLNL) cDNA Clones (Lennon, et al., *Genomics* 33: 151-52 (1996)). EST cDNA clone R25797 was sequenced to verify its homology to rat  
5 OAT1. The cDNA insert was excised by *NotI* and *HindIII* digestion and purified by agarose gel electrophoresis. These DNA fragments were then labeled with  $^{32}\text{P}$ - $\alpha$ -deoxyadenosine triphosphate (3000 Ci/mmol) using the Prime-a-Gene labeling system (Promega, Madison, WI) to a specific activity greater than  $1 \times 10^9$  dpm/ $\mu\text{g}$ .

A human kidney cDNA library in pTripleEx vector (Clontech, Palo Alto, CA) was  
10 plated out on 10 cm-diameter plates at a density of  $5 \times 10^4$  pfu/plate. The plates were incubated at 37°C for 6-8 hours until each plaque was about 0.3-0.5 mm in diameter. Plaque lifts were performed by placing a nylon membrane on each plate for 2 minutes. The nylon membrane was then lifted carefully from the plate and immersed in 1.5 M NaCl and 0.5 M NaOH for 2 minutes to denature the attached DNA. The nylon was then placed  
15 in 1.5 M NaCl and 0.5 M Tris (pH 8.0) for neutralization. Finally, the membrane was quickly rinsed in 2X SSC (0.3 M NaCl and 30 mM sodium citrate). The attached DNA was crosslinked by UV irradiation. Filters were incubated in hybridization buffer with radioactive probes at a concentration of  $1 \times 10^6$  dpm/ml in roller bottles at 65°C overnight. The next day, the filters were washed with 2X SSC and 0.1% SDS for 15 minutes at 25°C  
20 for two times. They were then washed with 0.2X SSC and 0.1% SDS for 1 hour at 65°C. After the final wash, filters were exposed to autoradiographic film with an intensifying screen at -80°C overnight. Positive clones, which show up as dark spots on the autorad, were identified and a 3 mm-diameter agar plug was removed from the original plate.

The positive clone was purified by subsequent rounds of hybridization until a well-  
25 isolated plaque could be obtained. The cDNA of interest was excised from the phage into a phagmid by an *in vivo* excision procedure using BM25.8 cells as host (Clontech, Palo Alto, CA). Phagmids were amplified, purified, and sequenced using standard procedures. The DNA sequence was analyzed for open reading frames and compared to published sequences.

30 One positive plaque isolated from the above procedure was 2.5 kb in length. Analysis of its sequence revealed an open-reading frame with a deduced amino acid sequence showing high homology to the rat OAT1 protein (Table 1). This clone was



designated human OAT1 (hOAT1), or the human homologue of rat OAT1. The entire cDNA and deduced amino acid sequences of human OAT1 are shown in Figure 1.

The isolated cDNA encoding for hOAT1 is predicted to encode a polypeptide with a length of 550 amino acids. Computer analysis of the deduced amino acid encoded by hOAT1 cDNA reveals consensus protein kinase C (PKC) phosphorylation sites at amino acids 271, 278, and 284.

Computer analysis using the GAP-Alignment program from the Wisconsin Genetics Software Package indicates that hOAT1 polypeptide is 88% identical to rat OAT1 polypeptide and 39% identical to rat OAT2 polypeptide (Table 1). GAP analysis also indicates that hOAT1 is 86% identical to rat OAT1 nucleotide sequence (Table 1).

EST accession number R25797 is homologous to hOAT1 cDNA at nucleotides 398-2100 (Figure 1).

**Table1**

| Homology Among Human and Rat Organic Anion Transporters |  |                 |                 |                |                |                |
|---|--|-----------------|-----------------|----------------|----------------|----------------|
|   | hOAT1<br>N <sup>1</sup> (A) <sup>2</sup> | hOAT2A<br>N (A) | hOAT2B<br>N (A) | hOAT3<br>N (A) | hOAT4<br>N (A) | hOAT5<br>N (A) |
| rat OAT1  | 86% (88%)                                | 53% (37%)       | 53% (37%)       | 60% (50%)      | 48% (36%)      | 51% (40%)      |
| rat OAT2  | 53% (39%)                                | 82% (79%)       | 82% (79%)       | 51% (37%)      | 44% (30%)      | 46% (31%)      |
| hOAT1   |  | 54% (39%)       | 55% (39%)       | 61% (51%)      | 49% (37%)      | 50% (40%)      |
| hOAT2A  |  |                 | 99% (98%)       | 52% (38%)      | 44% (32%)      | 45% (32%)      |
| hOAT2B  |  |                 |                 | 52% (39%)      | 44% (33%)      | 45% (32%)      |
| hOAT3   |  |                 |                 |                | 52% (37%)      | 52% (39%)      |
| hOAT4   |  |                 |                 |                |                | 72% (55%)      |
| hOAT5   |  |                 |                 |                |                |                |

1 (N) indicates the percentage of identical nucleotides found between compared nucleic acid sequences as determined using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weigh of 0.3.

2 (A) indicates the percentage of identical amino acids found between compared polypeptides as determined using the GAP-Alignment program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

Example 3: Isolation of full-length human OAT2A and OAT2B cDNA

Human OAT2 cDNA was cloned in the same manner as described for human OAT1 cDNA in Example 1 except that EST accession number-A1016020 was used as a probe. In addition, hOAT2A and hOAT2B were isolated from a cDNA library obtained  
5 from human liver rather than kidney, as was hOAT1.

The entire cDNA and deduced amino acid sequences of human OAT2A are shown in Figure 2. Human OAT2A (hOAT2A) cDNA is predicted to encode a polypeptide that is 546 amino acids in length. Computer analysis of the deduced amino acid sequence using the GAP-Alignment program indicates that human OAT2A polypeptide is 79%  
10 identical to rat OAT2 polypeptide (Table 1). GAP analysis also indicates that the nucleotide sequence for hOAT2A is 82% identical to the nucleotide sequence of rat OAT2.

EST accession number A1016020 is homologous to hOAT2A cDNA at nucleotide sequences 20-1400 (Figure 2).

15 The entire cDNA and deduced amino acid sequences of human OAT2B (hOAT2B) are shown in Figure 3. Human OAT2B cDNA is predicted to encode a polypeptide that is 538 amino acids in length. Human OAT2B is identical to hOAT2A except at its C-terminus end. Without wishing to be bound by any particular theory, hOAT2B could be an mRNA splice variant of hOAT2A.

20

Example 4: Isolation of full-length human OAT3 cDNA

Human OAT3 (hOAT3) cDNA was isolated as described for hOAT1 cDNA in Example 1 except that EST accession number-H41333 was used as a probe. The entire cDNA and deduced amino acid sequences of human OAT3 are shown in Figure 4.

25 Computer analysis of the hOAT3 cDNA sequence predicts that hOAT3 cDNA encodes a polypeptide that is 542 amino acids in length. Computer analysis of the deduced amino acid sequence reveals consensus PKC phosphorylation sites at amino acids 259, 266, 269, 511, and 527.

EST accession number H41333 is homologous to hOAT3 cDNA at nucleotides  
30 1169-2121 (Figure 4).

Example 5: Isolation of full-length human OAT4 cDNA

Human OAT4 (hOAT4) cDNA was cloned in the same manner as described for human OAT1 cDNA in Example 1 except that EST accession number-AA705512 was used as a probe. In addition, hOAT4 cDNA was isolated from a cDNA library obtained from human liver rather than kidney, as was hOAT1. The entire cDNA and deduced amino acid sequences of human OAT4 are shown in Figure 5. Computer analysis of human OAT4 cDNA predicts that hOAT4 cDNA encodes a polypeptide that is 554 amino acids in length. Computer analysis of the deduced amino acid sequence reveals a consensus PKC phosphorylation site at amino acid 324.

EST accession number AA705512 is homologous to hOAT4 cDNA at nucleotides 1-1232 (Figure 5).

Example 6: Isolation of full-length hOAT5 cDNA

Human OAT5 (hOAT5) cDNA was cloned in the same manner as described for human OAT1 cDNA in Example 1 except that EST accession number-AAA705512 was used as a probe. In addition, hOAT5 cDNA was isolated from a cDNA library obtained from human liver rather than kidney, as was hOAT1. The entire cDNA and deduced amino acid sequences of human OAT5 are shown in Figure 6. Human OAT5 cDNA is predicted to encode a polypeptide that is 541 amino acids in length. Computer analysis of the deduced amino acid sequence reveals consensus PKC phosphorylation sites at amino acids 282, 289, 345, and 526.

EST accession number AA705512 is homologous to hOAT5 cDNA at nucleotides 1-1193 (Figure 6).

Example 7: Functional expression of human OAT1 in HeLa cells

Human OAT1 cDNA was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA), which contains a cytomegalovirus promoter. The plasmid construct (pcDNA-hOAT1) was then amplified and purified. The construct was transfected into HeLa cells (ATCC), cultured in 25 mm<sup>2</sup> flasks, using the Effectene transfection reagent (Qiagen, Stanford Santa Clarita, CA). Control cell lines were transfected with a pcDNA-lacZ construct. One day post-transfection, the cells were split into six (6)-well plates and cultured for another twenty-four (24) hours. Transfected cells were tested for transport of <sup>3</sup>H-*p*-aminohippurate, a classical substrate of the kidney

organic anion transporter. Since OAT1 is an antiporter, the transfected cells were first preloaded with 1 mM glutarate for one (1) hour. The glutarate-containing media was removed and replaced with media containing 50  $\mu$ M PAH at 1  $\mu$ Ci/ml. After ten (10) minutes at 37°C, the media was aspirated and the cells were washed three times with ice cold phosphate-buffered saline (PBS). The cells were then solubilized with 0.5% SDS. The amount of labeled PAH transported into the cells was determined by a scintillation counter. As shown in Fig. 7, HeLa cells transfected with hOAT1 were able to uptake PAH whereas HeLa cells transfected with a vector control were only able to uptake a very small amount of PAH (almost 10-fold less than cells transfected with a vector containing DNA encoding hOAT1).

Example 8: Functional expression of hOAT1 in *Xenopus* oocytes

Complementary hOAT1 RNA was synthesized *in vitro* from the pcDNA-hOAT1 plasmid using T7 RNA polymerase (Ambion, Austin, TX). The transcription reaction was performed at 37°C for 2 hours. The RNA was purified by phenol and chloroform extraction and precipitated with ammonium acetate and ethanol. Purified RNA was analyzed for size and purity by agarose gel electrophoresis. RNA was resuspended in water at a concentration of 0.3  $\mu$ g/ $\mu$ l. Oocytes were prepared from ovaries removed from female *Xenopus laevis* according to White et al., (1985) *PNAS* 82:4852-56. About 15 ng of hOAT1 cRNA in 50 nl was injected into each oocyte. As a control, 50 nl of water was injected into each oocyte. The injected oocytes were assayed for transport of PAH two (2) days post-injection. Oocytes were preloaded with 1 mM glutarate for 2 hours in Barth buffer. Transport assays were conducted in modified Barth buffer (100 mM NaCl, 2mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, pH 7.5) with 50  $\mu$ M PAH at 4  $\mu$ Ci/ml. After a one (1) hour incubation at 25°C, oocytes were washed with 3 ml ice cold transport assay buffer three (3) times. Each oocyte was solubilized in 0.5 ml 0.5% SDS and subjected to scintillation counting. As shown in Fig. 8, oocytes injected with the hOAT1 RNA uptake <sup>3</sup>H-PAH to a much greater extent than water injected oocytes.

Example 9: Identification of fluorescein as a substrate for hOAT1

Human embryonic kidney (HEK293) cells were transfected with pcDNA-hOAT1, as in Example 7. One day after transfection the cells were assayed for transport of fluorescein or SITS. Ten (10)  $\mu$ M of either substrate was added to the external media for

ten (10) minutes at 37°C. The cells were washed with ice-cold PBS to remove excess label and analyzed under a fluorescent microscope. Cells that take up either fluorescein or SITS are predicted to turn fluorescent green. Fluorescent microscopy revealed that hOAT1-expressing cells took up fluorescein but not SITS.

5

Example 10: Competition assay to identify substrates of hOAT1 using fluorescein

HEK293 cells were transiently transfected with pcDNA3-hOAT1 as in Example 7 and assayed for transport of fluorescein, but in the presence or absence of 0.5 mM PAH. Since fluorescein and PAH are both substrates of hOAT1, they will compete for access to the transporter. As evidenced by fluorescent microscopy, hOAT1-expressing cells showed uptake of fluorescein in the absence but not in the presence of external PAH.

15

Example 11: Establishment of Cell lines stably expressing hOATs.

Vector pIND(sp1) (Invitrogen) contains the ecdysone-inducible promoter (EcRE). Using standard methods known to those in the art, cDNAs encoding hOAT1, hOAT2A, into vector pIND/sp1 downstream of its EcRE promoter. After cloning, the vectors were separately transfected into cell line EcR293 (Invitrogen). The EcR293 cell line is a derivative of cell line HEK 293 and contains a stably integrated pVgRXR vector. The pVgRXR vector constitutively expresses the ecdysone receptor and the retinoid X receptor (RXR). The ecdysone receptor and RXR form a heterodimer which, upon activation by the hormone ecdysone, binds to a promoter containing the EcRE. The pVgRXR vector was maintained within the EcR293 cell line by selection with 400 µg/ml zeocin. The pIND(sp1)-hOAT plasmids contain a neomycin resistance marker, and cells stably integrating the plasmids were selected for by culturing the cells in media supplemented with 400 µg/ml G418. After two weeks of selection, individual colonies appeared and were isolated and expanded. To test for expression of the transporter, the cells were induced with 1.25 µg/ml ponasterone, an ecdysone analogue, for twenty-four (24) hours. The cells were then subjected to standard transport assays.

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EcR293 cells with stably integrated pIND(sp1)-hOAT1 expressed hOAT1 at a basal level that was not harmful to the cells. These cells grow at a rapid rate comparable to the parental EcR293 cells. Hence, the hOAT1 cDNA can be maintained within these cells indefinitely. Expression of hOAT1 at high levels can be easily achieved by inducing

with ponasterone for twenty-four (24) hours. As shown in Fig. 9, ECR293 cells containing hOAT1 in plasmid pIND(sp-1) uptake approximately 50-fold more PAH when induced by ponasterone than non-induced cells containing pIND(sp-1)-hOAT1. ECR293 cells stably containing hOAT2A, in plasmid pIND(sp-1), uptake approximately 2.6-fold more <sup>3</sup>H-PAH when induced by ponasterone than non-induced cells (Fig. 10). Cell lines stably expressing hOAT2B, hOAT3, hOAT4, and hOAT5 can be established using the same method as for hOAT1 and hOAT2A.

**Example 12:** Competition assay to identify new substrates of hOAT2, using a radiolabeled substrate

EcR293/OAT2A cells were assayed for transport of <sup>3</sup>H-PAH as in Example 7. The uptake of <sup>3</sup>H-PAH was blocked by 100 μM sulfobromophthalein (BSP) but not 500 μM α-ketoglutarate (AKG) (Fig. 11). The competition assay thus identified BSP and not AKG as a preferred substrate of hOAT2A.

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**Example 13:** Predicting pharmacokinetics in man using a panel of hOAT expressing cells

A panel of different cell lines, each expressing a particular human OAT protein, can be used to predict pharmacokinetics for anionic drugs. For instance, PAH is a compound transported rapidly by hOAT1 and to a much lesser extent by the other human OATs disclosed in the invention. The panel of hOAT expressing cells would predict that PAH is eliminated rapidly by the human kidney, as previously described. Similarly, the competition assays in Examples 10 and 12 showed that fluorescein is a very good substrate of human OAT1 and that BSP is a good substrate of human OAT2A. Therefore, the assay would predict that fluorescein is eliminated from the human body mainly via kidney excretion and that BSP is mainly eliminated from the human body via liver excretion.

Further confirmation that a compound is a substrate of an hOAT would be done using *in vivo* tissue distribution assays. Compounds found to be transported by hOATs, using the cell line described above, would be injected into animals and followed to confirm their transport by the appropriate hOAT. For instance, a compound found to be transported by a cell line stably expressing hOAT1, would be expected to be excreted in the urine of an animal injected with the said compound. Similarly, a compound found to



be transported by a cell line expressing hOAT2, would be expected to be transported into the liver of animals injected with said compound.

Since hOAT4 and 5 have homology to OCTs, their range of substrates may include organic cations. Therefore, these transporters may be used to predict the pharmacokinetics of certain cationic drugs as well, using the methods described herein.

Example 14: Identification of hOAT substrates using antiporter activity.

EcR293/hOAT1 cells are incubated with  $^3\text{H}$ - $\alpha$ -ketoglutarate for an hour to preload with labeled  $\alpha$ -ketoglutarate. A test substrate is added to the cells. After a 10 minute incubation, an aliquot of the supernatant is removed and measured for effluxed  $\alpha$ -ketoglutarate by scintillation counting. Substrates of hOAT1 will show trans-stimulation of  $\alpha$ -ketoglutarate exchange. Inhibitors or non-substrates of hOAT1 will not stimulate  $\alpha$ -ketoglutarate efflux.

Example 15: Cytotoxicity assays using cell lines stably expressing hOAT polypeptides

EcR293 cells stably transformed with hOAT polypeptides can be used to screen for compounds potentially toxic to certain organs or tissues through use of a cytotoxicity assay. Test compounds can be added to cell lines stably expressing hOAT polypeptides. Compounds transported by hOAT polypeptides and which are toxic will cause cell death. Cell death can be measured or determined using techniques well known in the art, such as visual inspection, microscopic inspection, and dye exclusion. Using dye exclusion, healthy cells are able to exclude a particular dye whereas injured or dead cells are unable to exclude the dye.

The foregoing examples are not limiting and are merely representative of various aspects and features of the present invention. All references referred to above are incorporated herein by reference.

Example 16: Functional expression of hOAT3 in *Xenopus* oocytes

Complementary hOAT3 RNA was synthesized *in vitro* from the pcDNA-hOAT3 plasmid using T7 RNA polymerase (Ambion, Austin, TX). The transcription reaction was performed at 37°C for 2 hours. The RNA was purified by phenol and chloroform extraction and precipitated with ammonium acetate and ethanol. Purified RNA was analyzed for size and purity by agarose gel electrophoresis. RNA was resuspended in

water at a concentration of 0.5  $\mu\text{g}/\mu\text{l}$ . Oocytes were prepared from ovaries removed from female *Xenopus laevis* according to White et al., (1985) *PNAS* 82:4852-56. About 25 ng of hOAT3 cRNA in 50 nl was injected into each oocyte. As a control, 50 nl of water was injected into each oocyte. The injected oocytes were assayed for transport of PAH two (2) days post-injection. Transport assays were conducted in modified Barth buffer (100 mM NaCl, 2mM KCl, 1 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 10 mM HEPES, pH 7.5) with 50  $\mu\text{M}$  PAH at 10  $\mu\text{Ci}/\text{ml}$ . After a one (1) hour incubation at 25°C, oocytes were washed with 3 ml ice cold transport assay buffer three (3) times. Each oocyte was solubilized in 0.5 ml 0.5% SDS and subjected to scintillation counting. As shown in Fig. 12, oocytes injected with the hOAT3 RNA uptake  $^3\text{H}$ -PAH to a much greater extent than water injected oocytes.

Example 17: Determination of tissue distribution of hOATs

The individual hOAT cDNAs were labeled with  $^{32}\text{P}$ -ATP to a specific activity of  $> 1 \times 10^9$  dpm/ $\mu\text{g}$  using the Strip-EZ kit<sup>TM</sup> (Ambion). The Strip-EZ kit<sup>TM</sup> allows easy removal of probes from a blot and the blot can thus be reprobed several times without substantial loss of signal. The Human Multiple Tissue Northern blot was obtained from Clontech, which contained mRNA extracted from 12 different human tissues. The blot was successively hybridized with each hOAT probe in 5 ml of ExpressedHyb buffer (Clontech) at a final concentration of  $3\text{-}5 \times 10^6$  dpm/ml. Hybridization was carried out at 65°C for 4 hrs. The blot was washed two times with 2X SSC and 0.1% SDS at 65°C for 15 min each, and then once with 0.1X SSC and 0.1% SDS at 65°C for 1 hr. The blot was then exposed to BioMax MR autographic film (Kodak) with intensifying screen at -80°C for 24 hrs.

The northern analysis shows that hOAT1 was expressed only in human kidney (FIG 13). The OAT1 probe hybridized to a major band at about 2.4 kb. The hOAT2 probe hybridized to two major bands at about 2.4 and about 3 kb from liver and kidney, with higher levels in the liver than kidney (FIG 13). HOAT3 was expressed only in the kidney, with a major transcript of about 2.4 kb and minor transcript at about 4.3 kb (FIG 13). HOAT4 and hOAT5 were expressed exclusively in the liver (FIG 13). hOAT4 probe hybridized to a major band at about 4.3 kb and hOAT5 probe hybridized to a major band at about 2.5 kb. Hence, northern analyses indicated that hOAT1 and 3 are mainly kidney transporters and

hOAT4 and 5 are liver transporters, whereas hOAT2 is a liver and kidney transporter.

5 All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually. None of the references are admitted to be prior art.

10 One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

15 It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

20 The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, 25 and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

30 In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art

will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Other embodiments are within the following claims.

**What is claimed is:**

1. An isolated, enriched, or purified nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.
- 5 2. An isolated, enriched, or purified nucleic acid molecule comprising a nucleic acid molecule which encodes a human OAT polypeptide selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.
- 10 3. An isolated, enriched, or purified nucleic acid molecule which is complementary to any of the nucleic acid molecules of claims 1 and 2.
4. The isolated, enriched, or purified nucleic acid molecule of claim 1,  
15 wherein said nucleic acid molecule is SEQ ID NO:2.
5. The isolated, enriched, or purified nucleic acid molecule of claim 1,  
wherein said nucleic acid molecule is SEQ ID NO:3.
- 20 6. The isolated, enriched, or purified nucleic acid molecule of claim 1,  
wherein said nucleic acid molecule is SEQ ID NO:4.
7. The isolated, enriched, or purified nucleic acid molecule of claim 1,  
wherein said nucleic acid molecule is SEQ ID NO:5.
- 25 8. The isolated, enriched, or purified nucleic acid molecule of claim 1,  
wherein said nucleic acid molecule is SEQ ID NO:6.
9. The isolated, enriched, or purified nucleic acid molecule of claim 2,  
30 wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:8.
10. The isolated, enriched, or purified nucleic acid molecule of claim 2,  
wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:9.
11. The isolated, enriched, or purified nucleic acid molecule of claim 2,  
35 wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:10.

12. The isolated, enriched, or purified nucleic acid molecule of claim 2,  
wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:11.

13. The isolated, enriched, or purified nucleic acid molecule of claim 2,  
5 wherein said nucleic acid molecule encodes a polypeptide that is SEQ ID NO:12.

14. An isolated nucleic acid molecule comprising a nucleotide sequence  
selected from the group consisting of:

(a) a nucleotide sequence which encodes a naturally occurring variant  
10 of a polypeptide comprising the amino acid sequence of SEQ ID NO:8, wherein the  
nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:2 under  
stringent conditions;

(b) a nucleotide sequence which encodes a naturally occurring variant  
of a polypeptide comprising the amino acid sequence of SEQ ID NO:9, wherein the  
15 nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:3 under  
stringent conditions;

(c) a nucleotide sequence which encodes a naturally occurring variant  
of a polypeptide comprising the amino acid sequence of SEQ ID NO:10, wherein the  
nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:4 under  
20 stringent conditions;

(d) a nucleotide sequence which encodes a naturally occurring variant  
of a polypeptide comprising the amino acid sequence of SEQ ID NO:11, wherein the  
nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:5 under  
stringent conditions; and

25 (e) a nucleotide sequence which encodes a naturally occurring variant  
of a polypeptide comprising the amino acid sequence of SEQ ID NO:12, wherein the  
nucleotide sequence hybridizes to a nucleic acid molecule comprising SEQ ID NO:6 under  
stringent conditions.



15. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 83% identity to nucleotide sequence contained in SEQ ID NO:2, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

5

16. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 83% identity to nucleotide sequence contained in SEQ ID NO:3, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

10

17. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 61% identity to nucleotide sequence contained in SEQ ID NO:4, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

15

18. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 60% identity to nucleotide sequence contained in SEQ ID NO:5, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

20

19. An isolated nucleic acid molecule comprising a nucleotide sequence that has at least 60% identity to nucleotide sequence contained in SEQ ID NO:6, wherein the percent identity is calculated using the GAP-Alignment program in the GCG software package, using a gap weight of 5.0 and a length weight of 0.3.

25

20. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 80% percent identical to the amino acid sequence of SEQ ID NO:8 or 9, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

30

21. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 70% percent identical to the amino acid sequence of SEQ ID NO:10, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

5

22. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 70% percent identical to the amino acid sequence of SEQ ID NO:11, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

10

23. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide which is at least 70% percent identical to the amino acid sequence of SEQ ID NO:12, wherein said percent identity is calculated using the GAP program in the GCG software package, using a gap weight of 3.0 and a length weight of 0.1.

15

24. The nucleic acid molecule of claim 1 or 2, further comprising a vector or promoter effective to initiate transcription in a host cell.

25. The nucleic acid molecule of claim 24, wherein said promoter comprises an inducible promoter.

26. A nucleic acid probe for the detection, isolation, purification, enrichment, or amplification of a nucleic acid molecule encoding human OAT in a sample, wherein said nucleic acid probes are selected from the group of nucleic acid sequences set forth in, SEQ ID NO:2 from nucleotide 1370- 1638, SEQ ID NO: 3 from nucleotide 1370-1614, SEQ ID NO:4: from nucleotide 1-1107, SEQ ID NO: 5 from nucleotide 995-1662 and SEQ ID NO:6 from nucleotide 993-1623.

27. A nucleic acid probe for the detection, isolation, purification, enrichment, or amplification of a nucleic acid molecule encoding human OAT in a sample, wherein said nucleic acid probes are selected from the group of nucleic acid sequences encoding the polypeptide fragment selected from the group consisting of amino acids 132-136 of SEQ ID NO:8, amino acids 132-136 of SEQ ID NO:9, amino acids 112-116 of SEQ ID NO:10, amino acids 136-140 of SEQ ID NO:11 and amino acids 135-139 of SEQ ID NO:12.

35

28. A recombinant cell comprising a nucleic acid molecule encoding a human OAT polypeptide wherein said nucleic acid molecule is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.
- 5 29. The cell of claim 28, wherein said cell is stably transformed.
30. The cell of claim 28 or 29, wherein said cell is a mammalian cell.
- 10 31. The cell of claim 28 or 29, wherein said polypeptide is a fragment of the polypeptide encoded by the amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.
- 15 32. The cell of claim 28 or 29, wherein said nucleic acid is expressed from an inducible promoter.
33. The cell of claim 32, wherein said promoter is inducible with ecdysone.
- 20 34. An isolated, enriched, or purified human OAT polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.
- 25 35. The polypeptide of claim 34, wherein said polypeptide is a fragment of the polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.
- 30 36. The polypeptide of claim 34, wherein said polypeptide is isolated, purified, or enriched from a cell that comprises an endogenous nucleic acid molecule that encodes said polypeptide.

37. The polypeptide of claim 34, wherein said polypeptide is isolated, purified, or enriched from a cell that is transformed with a nucleic acid molecule that encodes said polypeptide.

5 38. The polypeptide of claim 34, wherein said polypeptide is chemically synthesized.

39. A labeled or unlabeled antibody or antibody fragment having specific-binding affinity to a human OAT polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

15 40. A hybridoma which produces an antibody having specific binding affinity to a human OAT polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

20 41. A method for identifying modulators of human OAT2A, human OAT2B, human OAT3, human OAT4, or human OAT5 polypeptide, said method comprising:

- (a) contacting said hOAT polypeptide with a test substance;
- (b) adding a substrate,
- (c) measuring the activity of said hOAT polypeptide; and
- 25 (d) if said substance decreases transport, determining whether said test substance is a modulator or competitive inhibitor of said hOAT polypeptide.

42. A method for identifying a modulator of human OAT2A, human OAT2B, human OAT3, human OAT4, or human OAT5 polypeptide in a cell, said method

30 comprising:

- (a) expressing an hOAT polypeptide in a cell;
- (b) contacting said cell with a test substance;
- (c) adding a substrate;
- (d) measuring the activity of said hOAT polypeptide; and

(e) if said substance decreases transport, determining whether said test substance is a modulator or competitive inhibitor of said hOAT.

43. A method to screen for substrates or inhibitors of human OAT2A, human OAT2B, human OAT3, human OAT4, and human OAT5 using a cell stably transformed with an hOAT nucleic acid molecule and a competition assay, said method comprising:

(a) adding a test substrate to a cell line expressing said human OAT polypeptides;

(b) adding a substrate known to be transported by said human OAT polypeptides in said cell line;

(c) measuring the ability of said test substrate to compete with the uptake of said substrate known to be transported by human OAT1, human OAT2A, human OAT2B, human OAT3, human OAT4, and human OAT5; and

(d) confirming whether said test substrate is an inhibitor or substrate.

44. The method of claim 43, wherein said test substrate and/or known substrate are fluorescent or conjugated with a fluorescent labeled.

45. The method of claim 44, wherein said assay is evaluated using high-throughput screening.

46. A method for identifying substrates of human OAT polypeptides, said method comprising:

(a) loading a cell line expressing an hOAT polypeptide with a substance known to be effluxed from the interior of said cell as part of the antiporter activity of said hOAT polypeptide

(b) adding a test substrate to said cell line;

(c) measuring the efflux of said antiporter substance; and

(d) determining if said test substrate affected the efflux of said antiporter substance.

47. The cell of claim 28 or 29, wherein said cell is a yeast cell.

48. The cell of claim 28 or 29, wherein said cell is an insect cell.

49. A method of preparing an hOAT polypeptide comprising:
- (a) culturing the recombinant cell of claim 29 under conditions that permit expression of the hOAT polypeptide; and
  - (b) isolating said polypeptide.

5

50. An amphibian oocyte containing a nucleic acid molecule encoding for a human OAT polypeptide according to claim 2.

51. The amphibian oocyte of claim 50 that is a *Xenopus laevis* oocyte.

10

52. The nucleic acid molecules of claim 1 or 2, wherein said nucleic acid molecules comprise one or more regions that encode an hOAT polypeptide or an hOAT polypeptide fragment, where the hOAT polypeptide or the hOAT polypeptide fragment is fused to a non-hOAT polypeptide or epitope tag.

15

53. The nucleic acid molecule of claim 52, wherein said non-hOAT polypeptide or epitope tag is selected from the group consisting of glutathione-S-transferase, green-fluorescent protein and an amino terminal tag composed of histidine residues.

20

54. A method to identify toxic compounds transported by hOAT polypeptides comprising:

- (a) expressing an hOAT polypeptide in cells;
- (b) adding a test substrate to said cells; and
- (c) determining if said cells are viable.

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CTGAGCTGACCTGACCCCCAAAGTGAAGGAGAAGCTGCAAGGGAAAAGGGAGGGACAGAT  
1 -----+-----+-----+-----+-----+-----+-----+ 60  
GACTCGACTGGAAGTGGGGTTTCACTTCCTCTTCGACGTTCCCTTTTCCCTCCCTGTCTA  
CAGGGAGACCGGGAAGAAGGAGGAGCAGCCAAGGAGGCTGCTGTCCCCCACAGAGCAG  
61 -----+-----+-----+-----+-----+-----+-----+ 120  
GTCCCTCTGGCCCCTTCTTCCTCCTCGTGGTTCCTCCGACGACAGGGGGGTGTCTCGTC  
CTCGGACTCAGCTCCCGGAGCAACCCAGCTGCGGAGGCAACGGCAGTGCTGCTCCTCCAG  
121 -----+-----+-----+-----+-----+-----+-----+ 180  
GAGCCTGAGTCGAGGGCCTCGTTGGGTGACGCCTCCGTTGCCGTACGACGAGGAGGTC  
CGAAGGACAGCAGGCAGGCAGACAGACAGAGGTCTGGGACTGGAAGGCCTCAGCCCCCA  
181 -----+-----+-----+-----+-----+-----+-----+ 240  
GCTTCCTGTCTCGTCCGTCCGTCTGTCTGTCTCCAGGACCCTGACCTTCCGGAGTCCGGGGT  
GCCACTGGGCTGGGCTGGCCCAATGGCCTTTAATGACCTCCTGCAGCAGGTGGGGGGTG  
241 -----+-----+-----+-----+-----+-----+-----+ 300  
CGGTGACCCGACCCGGACCGGGTTACCGGAAATTACTGGAGGACGTCCGCCACCCCCAC  
M A F N D L L Q Q V G G V -  
TCGGCCGCTTCCAGCAGATCCAGGTCACCCTGGTGGTCCTCCCCCTGCTCCTGATGGCTT  
301 -----+-----+-----+-----+-----+-----+-----+ 360  
AGCCGGCGAAGGTCTAGGTCCAGTGGGACCACCAGGAGGGGGACGAGGACTACCGAA  
G R F Q Q I Q V T L V V L P L L L M A S -  
CTCACAACACCCTGCAGAACTTCACTGCTGCCATCCCTACCCACCCTGCGCCCGCCTG  
361 -----+-----+-----+-----+-----+-----+-----+ 420  
GAGTGTGTGGGACGTCTTGAAGTGACGACCGTAGGGATGGGTGGTGACGGCGGGCGGAC  
H N T L Q N F T A A I P T H H C R P P A -  
CCGATGCCAACCTCAGCAAGAACGGGGGGCTGGAGGTCTGGCTGCCCCGGGACAGGCAGG  
421 -----+-----+-----+-----+-----+-----+-----+ 480  
GGCTACGGTTGGAGTCGTTCTTGGCCCCCGACCTCCAGACCGACGGGGCCCTGTCCGTCC  
D A N L S K N G G L E V W L P R D R Q G -  
GGCAGCCTGAGTCCTGCCTCCGCTTCACCTCCCCCGCAGTGGGGACTGCCCTTTCTCAATG  
481 -----+-----+-----+-----+-----+-----+-----+ 540  
CCGTCCGACTCAGGACGGAGGCGAAGTGGAGGGGGCTACCCCTGACGGGAAAGAGTTAC  
Q P E S C L R F T S P Q W G L P F L N G -  
GCACAGAAGCCAATGGCACAGGGGCCACAGAGCCCTGCACCGATGGCTGGATCTATGACA  
541 -----+-----+-----+-----+-----+-----+-----+ 600  
CGTGTCTTCGGTTACCGTGTCCCCGGTGTCTCGGGACGTGGCTACCGACCTAGATACTGT  
T E A N G T G A T E P C T D G W I Y D N -  
ACAGCACCTTCCCATCTACCATCGTGAUAGTGGGACCTTGTGTGCTCTCACAGGGCCC  
601 -----+-----+-----+-----+-----+-----+-----+ 660

*FIG. 1A*

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TGTCGTGGAAGGGTAGATGGTAGCACTGACTCACCTGGAACACACGAGAGTGTCCCGGG  
S T F P S T I V T E W D L V C S H R A L -

661 TACGCCAGCTGGCCCAGTCCTTGTACATGGTGGGGGTGCTGCTCGGAGCCATGGTGTTCG 720  
-----+-----+-----+-----+-----+-----+  
ATGCGGTGCGACCGGGTCAGGAACATGTACCACCCACGACGAGCCTCGGTACCACAAGC  
R Q L A Q S L Y M V G V L L G A M V F G -

721 GCTACCTTGCAGACAGGCTAGGCCGCCGGAAGGTACTCATCTTGAACCTACCTGCAGACAG 780  
-----+-----+-----+-----+-----+-----+  
CGATGGAACGTCTGTCCGATCCGGCGGCCTTCCATGAGTAGAACTTGATGGACGTCTGTC  
Y L A D R L G R R K V L I L N Y L Q T A -

781 CTGTGTCAGGGACCTGCGCAGCCTTCGCACCCAACCTCCCATCTACTGCGCCTTCCGGC 840  
-----+-----+-----+-----+-----+-----+  
GACACAGTCCCTGGACGCGTCGGAAGCGTGGGTGTAAGGGGTAGATGACGCGGAAGGCCG  
V S G T C A A F A P N F P I Y C A F R L -

841 TCCTCTCGGGCATGGCTCTGGCTGGCATCTCCCTCAACTGCATGACACTGAATGTGGAGT 900  
-----+-----+-----+-----+-----+-----+  
AGGAGAGCCCGTACCGAGACCGACCGTAGAGGGAGTTGACGTACTGTGACTTACACCTCA  
L S G M A L A G I S L N C M T L N V E W -

901 GGATGCCCATTCACACACGGGCCTGCGTGGGCACCTTGATTGGCTATGTCTACAGCCTGG 960  
-----+-----+-----+-----+-----+-----+  
CCTACGGGTAAGTGTGTGCCCGGACGCACCCGTGGAACCTAACCGATACAGATGTCCGACC  
M P I H T R A C V G T L I G Y V Y S L G -

961 GCCAGTTCCTCCTGGCTGGTGTGGCCTACGCTGTGCCCCACTGGCGCCACCTGCAGCTAC 1020  
-----+-----+-----+-----+-----+-----+  
CGGTCAAGGAGGACCGACACACCGGATGCGACACGGGGTGACCGCGGTGGACGTGATG  
Q F L L A G V A Y A V P H W R H L Q L L -

1021 TGGTCTCTGCGCCTTTTTTGCCTTCTTCATCTACTCCTGGTTCTTCATTGAGTCGGCCC 1080  
-----+-----+-----+-----+-----+-----+  
ACCAGAGACGCGGAAAAAACGGAAGAAGTAGATGAGGACCAAGAAGTAACTCAGCCGGG  
V S A P F F A F F I Y S W F F I E S A R -

1081 GCTGGCACTCCTCCTCCGGGAGGCTGGACCTCACCTGAGGGCCCTGCAGAGAGTCGCCC 1140  
-----+-----+-----+-----+-----+-----+  
CGACCGTGAGGAGGAGGCCCTCCGACCTGGAGTGGGACTCCCGGGACGTCTCTCAGCGGG  
W H S S S G R L D L T L R A L Q R V A R -

1141 GGATCAATGGGAAGCGGGAAGAAGGAGCCAAATTGAGTATGGAGGTACTCCGGGCCAGTC 1200  
-----+-----+-----+-----+-----+-----+

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CCTAGTTACCCTTCGCCCTTCTTCCTCGGTTTAACTCATACCTCCATGAGGCCCGGTGAG  
I N G K R E E G A K L S M E V L R A S L -  
TGCAGAAGGAGCTGACCATGGGCAAAGGCCAGGCATCGGCCATGGAGCTGCTGCGCTGCC  
1201 -----+-----+-----+-----+-----+-----+-----+ 1260  
ACGTCTTCCTCGACTGGTACCCGTTTCCGGTCCGTAGCCGGTACCTCGACGACGCGACGG  
Q K E L T M G K G Q A S A M E L L R C P -  
CCACCCTCCGCCACCTCTTCCTCTGCCTCTCCATGCTGTGGTTTGCCACTAGCTTTGCAT  
1261 -----+-----+-----+-----+-----+-----+-----+ 1320  
GGTGGGAGGCGGTGGAGAAGGAGACGGAGAGGTACGACACCAAACGGTGATCGAAACGTA  
T L R H L F L C L S M L W F A T S F A Y -  
ACTATGGGCTGGTCATGGACCTGCAGGGCTTTGGAGTCAGCATCTACCTAATCCAGGTGA  
1321 -----+-----+-----+-----+-----+-----+-----+ 1380  
TGATACCCGACCAGTACCTGGACGTCCCGAAACCTCAGTCGTAGATGGATTAGGTCCACT  
Y G L V M D L Q G F G V S I Y L I Q V I -  
TCTTTGGTGCTGTGGACCTGCCTGCCAAGCTTGTGGGCTTCCTTGTTCATCAACTCCCTGG  
1381 -----+-----+-----+-----+-----+-----+-----+ 1440  
AGAAACCACGACACCTGGACGGACGGTTCGAACACCCGAAGGAACAGTAGTTGAGGGACC  
F G A V D L P A K L V G F L V I N S L G -  
GTCGCCGGCCTGCCCAGATGGCTGCACTGCTGCTGGCAGGCATCTGCATCCTGCTCAATG  
1441 -----+-----+-----+-----+-----+-----+-----+ 1500  
CAGCGGCCGGACGGGTCTACCGACGTGACGACGACCGTCCGTAGACGTAGGACGAGTTAC  
R R P A Q M A A L L L A G I C I L L N G -  
GGGTGATACCCAGGACCAGTCCATTGTCCGAACCTCTCTTGCTGTGCTGGGGAAGGGTT  
1501 -----+-----+-----+-----+-----+-----+-----+ 1560  
CCCCTATGGGGTCCTGGTCAGGTAAACAGGCTTGGAGAGAACGACACGACCCCTTCCCAA  
V I P Q D Q S I V R T S L A V L G K G C -  
GTCTGGCTGCCTCCTTCAACTGCATCTTCCTGTATACTGGGGAAGTGTATCCCACAATGA  
1561 -----+-----+-----+-----+-----+-----+-----+ 1620  
CAGACCGACGGAGGAAGTTGACGTAGAAGGACATATGACCCCTTGACATAGGGTGTTACT  
L A A S F N C I F L Y T G E L Y P T M I -  
TCCGGCAGACAGGCATGGGAATGGGCAGCACCATGGCCCGAGTGGGCAGCATCGTGAGCC  
1621 -----+-----+-----+-----+-----+-----+-----+ 1680  
AGGCCGTCTGTCCGTACCCCTTACCCGTCTGGTACCGGGCTCACCCGTCGTAGCACTCGG  
R Q T G M G M G S T M A R V G S I V S P -  
CACTGGTGAGCATGACTGCCGAGCTCTACCCCTCCATGCCTCTCTTCATCTACGGTGCTG  
1681 -----+-----+-----+-----+-----+-----+-----+ 1740

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GTGACCACTCGTACTGACGGCTCGAGATGGGGAGGTACGGAGAGAAGTAGATGCCACGAC  
L V S M T A E L Y P S M P L F I Y G A V -

1741 TTCCTGTGGCCGCCAGCGCTGTCCTGTCCTGCCAGAGACCCTGGGCCAGCCACTGC 1800  
-----+-----+-----+-----+-----+-----+  
AAGGACACCGGCGGTGCGGACAGTGACAGGAGGACGGTCTCTGGGACCCGGTCGGTGACG  
P V A A S A V T V L L P E T L G Q P L P -

1801 CAGACACGGTGCAGGACCTGGAGAGCAGGAAAGGGAAACAGACGCGACAGCAACAAGAGC 1860  
-----+-----+-----+-----+-----+-----+  
GTCTGTGCCACGTCTGGACCTCTCGTCCTTTCCCTTTGTCTGCGCTGTCGTTGTTCTCG  
D T V Q D L E S R K G K Q T R Q Q Q E H -

1861 ACCAGAAGTATATGGTCCCACTGCAGGCCTCAGCACAAGAGAAGAATGGACTCTGAGGAC 1920  
-----+-----+-----+-----+-----+-----+  
TGGTCTTCATATAACCAGGGTGACGTCCGGAGTCGTGTTCTCTTCTTACCTGAGACTCCTG  
Q K Y M V P L Q A S A Q E K N G L \*

1921 TGAGAAGGGGCCTTACAGAACCCTAAAGGGAGGGAAGGTCCTACAGGTCTCCGGCCACCC 1980  
-----+-----+-----+-----+-----+-----+  
ACTCTTCCCCGGAATGTCTTGGGATTTCCTCCCTTCCAGGATGTCCAGAGGCCGGTGGG

1981 ACACAAGGAGGAGGAAGAGGAAATGGTGACCCAAGTGTGGGGGTGTGGTTCAGGAAAGC 2040  
-----+-----+-----+-----+-----+-----+  
TGTGTTCTCCTCCTTCTCCTTTACCACTGGGTTACACCCCCAACACCAAGTCCTTTTCG

2041 ATCTTCCCAGGGGTCCACCTCCCTTTATAAACCCACCAGAACCACATCATTAAAAGGTT 2100  
-----+-----+-----+-----+-----+-----+  
TAGAAGGGTCCCAGGTGGAGGGAAATATTTGGGGTGGTCTTGGTGTAGTAATTTTCCAA

2101 TGACTGCGCACCAAAAAAAAAAAAAAAAAA 2100  
-----+-----+-----+  
ACTGACGCGTGGTTTTTTTTTTTTTTTTT

*FIG. 1D*

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1 CTGCACCTGAAGCATTGTTGGTGGGTGAGCAGCATGGGCTTTGAGGAGCTGCTGGAGCAGGT 60  
-----+-----+-----+-----+-----+-----+  
GACGTGGACTTCGTAAACCACCCACTCGTCGTACCCGAACTCCTCGACGACCTCGTCCA  
M G F E E L L E Q V -

61 GGGCGGCTTTGGGCCCTTCCAACCTGCGGAATGTGGCACTGCTGGCCCTGCCCCGAGTGCT 120  
-----+-----+-----+-----+-----+-----+  
CCCGCCGAAACCCGGGAAGGTTGACGCCTTACACCGTGACGACCGGGACGGGGCTCACGA  
G G F G P F Q L R N V A L L A L P R V L -

121 GCTACCACTGCACTTCCTCCTGCCATCTTCCTGGCTGCCGTGCCTGCCACCGATGTGC 180  
-----+-----+-----+-----+-----+-----+  
CGATGGTGACGTGAAGGAGGACGGGTAGAAGGACCGACGGCACGGACGGGTGGCTACACG  
L P L H F L L P I F L A A V P A H R C A -

181 CCTGCCGGGTGCCCTGCCAACTTCAGCCATCAGGATGTGTGGCTGGAGGCCCATCTTCC 240  
-----+-----+-----+-----+-----+-----+  
GGACGGGCCACGGGGACGGTTGAAGTCGGTAGTCCTACACACCGACCTCCGGGTAGAAGG  
L P G A P A N F S H Q D V W L E , A H L P -

241 CCGGGAGCCTGATGGCACGCTCAGCTCCTGCCTCCGCTTTGCCTATCCCCAGGCTCTCCC 300  
-----+-----+-----+-----+-----+-----+  
GGCCCTCGGACTACCGTGCGAGTCGAGGACGGAGGCGAAACGGATAGGGGTCCGAGAGGG  
R E P D G T L S S C L R F A Y P Q A L P -

301 CAACACCACGTTGGGGGAAGAAAGGCAGAGCCGTGGGGAGCTGGAGGATGAACCTGCCAC 360  
-----+-----+-----+-----+-----+-----+  
GTTGTGGTGCAACCCCTTCTTTCCGTCTCGGCACCCCTCGACCTCCTACTTGGACGGTG  
N T T L G E E R Q S R G E L E D E P A T -

361 AGTGCCCTGCTCTCAGGGCTGGGAGTACGACCACTCAGAATTCTCCTCTACCATGCAAC 420  
-----+-----+-----+-----+-----+-----+  
TCACGGGACGAGAGTCCCGACCCTCATGCTGGTGAGTCTTAAGAGGAGATGGTAACGTTG  
V P C S Q G W E Y D H S E F S S T I A T -

421 TGAGTGGGATCTGGTGTGTGAGCAGAAAGGTCTGAACAGAGCTGCGTCCACTTTCTTCTT 480  
-----+-----+-----+-----+-----+-----+  
ACTCACCCTAGACCACACACTCGTCTTTCCAGAC TTGTCTCGACGCAGGTGAAAGAAGAA  
E W D L V C E Q K G L N R A A S T F F F -

481 CGCCGGTGTGCTGGTGGGGGCTGTGGCCTTTGGATATCTGTCCGACAGGTTTGGGCGGCG 540  
-----+-----+-----+-----+-----+-----+  
GCGGCCACACGACCACCCCGACACCGGAAACCTATAGACAGGCTGTCCAAACCCGCCGC  
A G V L V G A V A F G Y L S D R F G R R -

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541 GCGTCTGCTGCTGGTAGCCTACGTGAGTACCCTGGTGCTGGGCCTGGCATCTGCAGCCTC 600  
-----+-----+-----+-----+-----+-----+  
CGCAGACGACGACCATCGGATGCACTCATGGGACCACGACCCGGACCGTAGACGTCCGAG  
R L L L V A Y V S T L V L G L A S A A S -  
601 CGTCAGCTATGTAATGTTTGCCATCACCCGCACCCTTACTGGCTCAGCCCTGGCTGGTTT 660  
-----+-----+-----+-----+-----+-----+  
GCAGTCGATACATTACAAACGGTAGTGGGCGTGGAATGACCGAGTCGGGACCGACCAA  
V S Y V M F A I T R T L T G S A L A G F -  
661 TACCATCATCGTGATGCCACTGGAGCTGGAGTGGCTGGATGTGGAGCACCGCACCGTGGC 720  
-----+-----+-----+-----+-----+-----+  
ATGGTAGTAGCACTACGGTGACCTCGACCTCACCGACCTACACCTCGTGGCGTGGCACCG  
T I I V M P L E L E W L D V E H R T V A -  
721 TGGAGTCCTGAGCAGCACCTTCTGGACAGGGGGCGTGATGCTGCTGGCACTGGTTGGGTA 780  
-----+-----+-----+-----+-----+-----+  
ACCTCAGGACTCGTCGTGGAAGACCTGTCCCCGCACTACGACGACCGTGACCAACCCAT  
G V L S S T F W T G G V M L L A L V G Y -  
781 CCTGATACGGGACTGGCGATGGCTTCTGCTAGCTGTCACCCTGCCTTGTGCCCCAAGCAT 840  
-----+-----+-----+-----+-----+-----+  
GGACTATGCCCTGACCGCTACCGAAGACGATCGACAGTGGGACGGAACACGGGGTTCGTA  
L I R D W R W L L L A V T L P C A P S I -  
841 CCTCAGCCTCTGGTGGGTGCCTGAGTCTGCACGCTGGCTTCTGACCCAAGGCCATGTGAA 900  
-----+-----+-----+-----+-----+-----+  
GGAGTCGGAGACCACCCACGGACTCAGACGTGCGACCGAAGACTGGGTTCGGGTACACTT  
L S L W W V P E S A R W L L T Q G H V K -  
901 AGAGGCCACAGGTACTTGCTCCACTGTGCCAGGCTCAATGGGCGGCCAGTGTGTGAGGA 960  
-----+-----+-----+-----+-----+-----+  
TCTCCGGGTGTCCATGAACGAGGTGACACGGTCCGAGTTACCCGCCGGTCACACACTCCT  
E A H R Y L L H C A R L N G R P V C E D -  
961 CAGCTTCAGCCAGGAGGCTGTGAGCAAAGTGGCCGCCGGGGAACGGGTGGTCCGAAGACC 1020  
-----+-----+-----+-----+-----+-----+  
GTCGAAGTCGGTCCCTCCGACACTCGTTTCACCGGCGGCCCCCTTGCCACCGAGGCTTCTGG  
S F S Q E A V S K V A A G E R V V R R P -  
1021 TTCATACCTAGACCTGTTCCGCACACCACGGCTCCGACACATCTCACTGTGCTGCGTGGT 1080  
-----+-----+-----+-----+-----+-----+  
AAGTATGGATCTGGACAAGGCGTGTGGTGCCGAGGCTGTGTAGAGTGACACGACGCACCA  
S Y L D L F R T P R L R H I S L C C V V -

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1081 GGTGTGGTTCGGAGTGAACCTTCTCCTATTACGGCCTGAGTCTGGATGTGTCTGGGGCTGGG  
-----+-----+-----+-----+-----+-----+-----+ 1140  
CCACACCAAGCCTCACTTGAAGAGGATAATGCCGGACTCAGACCTACACAGCCCCGACCC  
V W F G V N F S Y Y G L S L D V S G L G -

1141 GCTGAACGTGTACCAGACACAGCTGTTGTTCTGGGGCTGTGGAAGTGCCTCCAAGCTGCT  
-----+-----+-----+-----+-----+-----+-----+ 1200  
CGACTTGACATGGTCTGTGTCTGACAACAAGCCCCGACACCTTGACGGGAGGTTGACGA  
L N V Y Q T Q L L F G A V E L P S K L L -

1201 GGTCTACTTGTCTGGTGTCTACGCAGGACGCCGCTCACGCAAGCCGGGACACTGCTGGG  
-----+-----+-----+-----+-----+-----+-----+ 1260  
CCAGATGAACAGCCACGCGATGCGTCCTGCGGCGGAGTGCCTTCGGCCCTGTGACGACCC  
V Y L S V R Y A G R R L T Q A G T L L G -

1261 CACGGCCCTGGCGTTCGGCACTAGACTGCTAGTGTCTCCGATATGAAGTCTGGAGCAC  
-----+-----+-----+-----+-----+-----+-----+ 1320  
GTGCCGGGACCGCAAGCCGTGATCTGACGATCACAGGAGGCTATACTTCAGGACCTCGTG  
T A L A F G T R L L V S S D M K S W S T -

1321 TGTCTGGCAGTGATGGGGAAAGCTTTTCTGAAGCTGCCTTCACCACTGCCTACCTGTT  
-----+-----+-----+-----+-----+-----+-----+ 1380  
ACAGGACCGTCACTACCCCTTTCGAAAAAGACTTCGACGGAAGTGGTGACGGATGGACAA  
V L A V M G K A F S E A A F T T A Y L F -

1381 CACTTCAGAGTTGTACCCTACGGTGCTCAGACAGACAGGGATGGGGCTGACTGCACTGGT  
-----+-----+-----+-----+-----+-----+-----+ 1440  
GTGAAGTCTCAACATGGGATGCCACGAGTCTGTCTGTCCCTACCCCGACTGACGTGACCA  
T S E L Y P T V L R Q T G M G L T A L V -

1441 GGGCCGGCTGGGGGGCTCTTTGGCCCCACTGGCGGCCTTGCTGGATGGAGTGTGGCTGTC  
-----+-----+-----+-----+-----+-----+-----+ 1500  
CCCGGCCGACCCCCCGAGAAACCGGGGTGACCGCCGGAACGACCTACCTCACACCGACAG  
G R L G G S L A P L A A L L D G V W L S -

1501 ACTGCCCAAGCTTACTTATGGGGGGATCGCCCTGCTGGCTGCCGGCACCGCCCTCCTGCT  
-----+-----+-----+-----+-----+-----+-----+ 1560  
TGACGGGTTCGAATGAATACCCCCCTAGCGGGACGACCGACGGCCGTGGCGGGAGGACGA  
L P K L T Y G G I A L L A A G T A L L L -

1561 GCCAGAGACGAGGCAGGCACAGCTGCCAGAGACCATCCAGGACGTGGAGAGAAAGAGTGC  
-----+-----+-----+-----+-----+-----+-----+ 1620  
CGGTCTCTGCTCCGTCCGTGTCTGACGGTCTCTGGTGGTCTGCACCTCTCTTTCTCAG  
P E T R Q A Q L P E T I Q D V E R K S A -

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1621 CCCAACCAGTCTTCAGGAGGAAGAGATGCCCATGAAGCAGGTCCAGAACTAAGTGGGAGT 1680  
-----+-----+-----+-----+-----+-----+  
GGGTTGGTCAGAAGTCCTCCTTCTCTACGGGTACTTCGTCCAGGTCTTGATTCACCCTCA  
P T S L Q E E E M P M K Q V Q N \*  
1681 GGAGGCAGGCCCTCCACAGAAGCTCTGCAGCAGGGGCTGGGAGAGCAGAAGGGCAGGCCC 1740  
-----+-----+-----+-----+-----+-----+  
CCTCCGTCCGGGAGGTGTCTTCGAGACGTCGTCCCCGACCCTCTCGTCTTCCCGTCCGGG  
1741 TGCAACTCAGGCTGGGAGTATCGAACCCTCTGCCTAGGGCCGGAGTTGCTGCCAGTACCC 1800  
-----+-----+-----+-----+-----+-----+  
ACGTTGAGTCCGACCCTCATAGCTTGGGAGACGGATCCCGGCCTCAACGACGGTCATGGG  
1801 GCTCCCTCTGCTCATCCATCCTTGATTATTTGGCTTCTAGGAACAGTTGACTTCCCAGAA 1860  
-----+-----+-----+-----+-----+-----+  
CGAGGGAGACGAGTAGGTAGGAACTAATAAACCGAAGATCCTTGCTCAACTGAAGGGTCTT  
1861 TGCAGTGGGCTGCTGGGCACCCCTCTCACGGTTGGGGAGGATTCTGTAAATAAAGGTGCC 1920  
-----+-----+-----+-----+-----+-----+  
ACGTCACCCGACGACCCGTGGGGAGAGTGCCAACCCCTCCTAAGACATTTATTTCCACGG  
1921 CCTTGGGTTGGGGCAATGGTGACGAGCTGTGGGAAGAGCCCTGGATAGGAAGCCACTGAG 1980  
-----+-----+-----+-----+-----+-----+  
GGAACCCAACCCCGTTACCACTGCTCGACACCCTTCTCGGGACCTATCCTTCGGTGACTC  
1981 TCTGCCCTGGGCTCTGATAAAACCTTCACCATTAACCTTGCTGTGTGACCTTGGGCATGTG 2040  
-----+-----+-----+-----+-----+-----+  
AGACGGGACCCGAGACTATTTTGGAGTGGTAATTGAACGACACACTGGAACCCGTACAC  
2041 GCTTTCCCTCTCTGGCCTCAGTCTGTTTCATCTCCCAAATGGATAATGAAGCCTCTTGGGA 2100  
-----+-----+-----+-----+-----+-----+  
CGAAAGGGAGAGACCGGAGTCAGACAAGTAGAGGGTTTACCTATTACTTCGGAGAACCCT  
2101 GGCCCTACCATAGGATCTGTTGCCATGCTCAAATGAGTTACTGAATAAGGTGCTTCTGCT 2160  
-----+-----+-----+-----+-----+-----+  
CCGGGATGGTATCCTAGACAACGGTACGAGTTTACTCAATGACTTATTCCACGAAGACGA  
2161 TCTTCTAGAGATGGTGCTAAAGAAAGGACTAGCATATGAGACTTCTGGTACCAATGGGGC 2220  
-----+-----+-----+-----+-----+-----+  
AGAAGATCTCTACCACGATTTCTTTCTGATCGTATACTCTGAAGACCATGGTTACCCCG  
2221 TGGTGGGCATGCTGTCCACTGTGTGGTGCTAGGAC TGCCAATGCCAGGCCCAAGGGACAA 2280  
-----+-----+-----+-----+-----+-----+  
ACCACCCGTACGACAGGTGACACACCACGATCCTGACGGTTACGGTCCGGGTTCCTGT  
2281 AAAGAACAGAGCTTTTGTTCATGGCTGGCCCTGCTACCTCCGAGGCACCCTGCAGGG 2340  
-----+-----+-----+-----+-----+-----+  
TTTCTTGTCTCGAAAAACAAGAGTACCGACCGGGACGATGGAGGCTCCGTGGGACGTCCC  
2341 CAATGCATGTCATCCCAACCCCCACACTCCCCATCTCCAACCCACTGGTCTCATGCCCA 2400  
-----+-----+-----+-----+-----+-----+  
GTTACGTACAGTAGGGTTGGGGGTGTGAGGGGTAGGAGGTTGGGTGACCAGAGTACGGGT

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2401 AAGAAGAGTTGAAGGCATGGGAGCCAACATTTTATTGAAGAAGCCACAGAGGCTGAAATT 2460  
-----+-----+-----+-----+-----+-----+  
TTCTTCTCAACTTCCGTACCCTCGGTTGTAAAATAACTTCTTCGGTGTCTCCGACTTTAA  
2461 CAATAAACACAAGTTTTATGAGTAAAAAAAAAAAAAAAAAAAA  
-----+-----+-----+-----+-----+ 2501  
GTTATTTGTGTTCAAATACTCATTTTTTTTTTTTTTTTTT

*FIG. 2E*

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1 CTGCACCTGAAGCATTGTTGGTGGGTGAGCAGCATGGGCTTTGAGGAGCTGCTGGAGCAGGT 60  
-----+-----+-----+-----+-----+-----+-----+  
GACGTGGACTTCGTAAACCACCCACTCGTCGTACCCGAAACTCCTCGACGACCTCGTCCA  
M G F E E L L E Q V -

61 GGGCGGCTTTGGGCCCTTCCAACCTGCGGAATGTGGCACTGCTGGCCCTGCCCCGAGTGCT 120  
-----+-----+-----+-----+-----+-----+-----+  
CCCGCCGAAACCCGGGAAGGTTGACGCCTTACACCGTGACGACCGGGACGGGGCTCACGA  
G G F G P F Q L R N V A L L A L P R V L -

121 GCTACCACTGCACTTCCTCCTGCCCATCTTCTGGCTGCCGTGCCTGCCACCGATGTGC 180  
-----+-----+-----+-----+-----+-----+-----+  
CGATGGTGACGTGAAGGAGGACGGGTAGAAGGACCGACGGCACGGACGGGTGGCTACACG  
L P L H F L L P I F L A A V P A H R C A -

181 CCTGCCGGGTGCCCTGCCAACTTCAGCCATCAGGATGTGTGGCTGGAGGCCCATCTTCC 240  
-----+-----+-----+-----+-----+-----+-----+  
GGACGGCCACGGGGACGGTTGAAGTCGGTAGTCCTACACACCGACCTCCGGGTAGAAGG  
L P G A P A N F S H Q D V W L E A H L P -

241 CCGGGAGCCTGATGGCACGCTCAGCTCCTGCCCTCCGCTTTGCCTATCCCCAGGCTCTCCC 300  
-----+-----+-----+-----+-----+-----+-----+  
GGCCCTCGGACTACCGTGCGAGTCGAGGACGGAGGCGAAACGGATAGGGGTCCGAGAGGG  
R E P D G T L S S C L R F A Y P Q A L P -

301 CAACACCACGTTGGGGGAAGAAAGGCAGAGCCGTGGGGAGCTGGAGGATGAACCTGCCAC 360  
-----+-----+-----+-----+-----+-----+-----+  
GTTGTGGTGCAACCCCTTCTTTCCGTCTCGGCACCCCTCGACCTCCTACTTGGACGGTG  
N T T L G E E R Q S R G E L E D E P A T -

361 AGTGCCCTGCTCTCAGGGCTGGGAGTACGACCACTCAGAATTCTCCTCTACCATTGCAAC 420  
-----+-----+-----+-----+-----+-----+-----+  
TCACGGGACGAGAGTCCCGACCCTCATGCTGGTGAGTCTTAAGAGGAGATGGTAACGTTG  
V P C S Q G W E Y D H S E F S S T I A T -

421 TGAGTGGGATCTGGTGTGTGAGCAGAAAGGTCTGAACAGAGCTGCGTCCACTTTCTTCTT 480  
-----+-----+-----+-----+-----+-----+-----+  
ACTCACCTAGACCACACACTCGTCTTTCCAGACTTGTCTCGACGCAGGTGAAAGAAGAA  
E W D L V C E Q K G L N R A A S T F F F -

481 CGCCGGTGTGCTGGTGGGGGCTGTGGCCTTTGGATATCTGTCCGACAGGTTTGGGCGGCG 540  
-----+-----+-----+-----+-----+-----+-----+  
GCGGCCACACGACCACCCCGACACCGGAAACCTATAGACAGGCTGTCAAACCCGCCGC  
A G V L V G A V A F G Y L S D R F G R R -

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541 GCGTCTGCTGCTGGTAGCCTACGTGAGTACCCTGGTGGCTGGGCCTGGCATCTGCAGCCTC 600  
-----+-----+-----+-----+-----+-----+  
CGCAGACGACGACCATCGGATGCACTCATGGGACCACGACCCGGACCGTAGACGTGCGGAG  
R L L L V A Y V S T L V L G L A S A A S -

601 CGTCAGCTATGTAATGTTTGCCATCACCCGCACCCTTACTGGCTCAGCCCTGGCTGGTTT 660  
-----+-----+-----+-----+-----+-----+  
GCAGTCGATACATTACAAACGGTAGTGGGCGTGGGAATGACCGAGTCGGGACCGACCAAA  
V S Y V M F A I T R T L T G S A L A G F -

661 TACCATCATCGTGATGCCACTGGAGCTGGAGTGGCTGGATGTGGAGCACCGCACCGTGGC 720  
-----+-----+-----+-----+-----+-----+  
ATGGTAGTAGCACTACGGTGACCTCGACCTCACCGACCTACACCTCGTGGCGTGGCACCG  
T I I V M P L E L E W L D V E H R T V A -

721 TGGAGTCCTGAGCAGCACCTTCTGGACAGGGGGCGTGATGCTGCTGGCACTGGTTGGGTA 780  
-----+-----+-----+-----+-----+-----+  
ACCTCAGGACTCGTCGTGGAAGACCTGTCCCCGCACTACGACGACCGTGACCAACCCAT  
G V L S S T F W T G G V M L L A L V G Y -

781 CCTGATACGGGACTGGCGATGGCTTCTGCTAGCTGTCACCCTGCCTTGTGCCCCAAGCAT 840  
-----+-----+-----+-----+-----+-----+  
GGACTATGCCCTGACCGCTACCGAAGACGATCGACAGTGGGACGGAACACGGGGTTCGTA  
L I R D W R W L L L A V T L P C A P S I -

841 CCTCAGCCTCTGGTGGGTGCCTGAGTCTGCACGCTGGCTTCTGACCCAAGGCCATGTGAA 900  
-----+-----+-----+-----+-----+-----+  
GGAGTCGGAGACCACCCACGGAAGACTCGACAGTGGGACGGAACACGGGGTTCGTA  
L S L W W V P E S A R W L L T Q G H V K -

901 AGAGGCCACAGGTAAGTCTGCTCCACTGTGCCAGGCTCAATGGGCGGCCAGTGTGTGAGGA 960  
-----+-----+-----+-----+-----+-----+  
TCTCCGGGTGTCCATGAACGAGGTGACACGGTCCGAGTTACCCGCCGGTCACACACTCCT  
E A H R Y L L H C A R L N G R P V C E D -

961 CAGCTTCAGCCAGGAGGCTGTGAGCAAAGTGGCCGCCGGGGAACGGGTGGTCCGAAGACC 1020  
-----+-----+-----+-----+-----+-----+  
GTCGAAGTCGGTCTCCGACACTCGTTTCACCGGCGGCCCTTGCCACCAAGGCTTCTGG  
S F S Q E A V S K V A A G E R V V R R P -

1021 TTCATACCTAGACCTGTTCCGCACACCACGGCTCCGACACATCTCACTGTGCTGCGTGGT 1080  
-----+-----+-----+-----+-----+-----+  
AAGTATGGATCTGGACAAGGCGTGTGGTGCCGAGGCTGTGTAGAGTGACACGACGCCA  
S Y L D L F R T P R L R H I S L C C V V -

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1081 GGTGTGGTTCGGAGTGAACCTTCTCCTATTACGGCCTGAGTCTGGATGTGTCTGGGGCTGGG 1140  
-----+-----+-----+-----+-----+-----+  
CCACACCAAGCCTCACTTGAAGAGGATAATGCCGGACTCAGACCTACACAGCCCCGACCC  
V W F G V N F S Y Y G L S L D V S G L G -

1141 GCTGAACGTGTACCAGACACAGCTGTTGTTCTGGGGCTGTGGAAGTGCCTCCAAGCTGCT 1200  
-----+-----+-----+-----+-----+-----+  
CGACTTGCACATGGTCTGTGTCTGACAACAAGCCCCGACACCTTGACGGGAGGTTTCGACGA  
L N V Y Q T Q L L F G A V E L P S K L L -

1201 GGTCTACTTGTCTGGTGGCTACGCAGGACGCCGCTCACGCAAGCCGGGACACTGCTGGG 1260  
-----+-----+-----+-----+-----+-----+  
CCAGATGAACAGCCACGCGATGCGTCTGCGGCGGAGTGCGTTCGGCCCTGTGACGACCC  
V Y L S V R Y A G R R L T Q A G T L L G -

1261 CACGGCCCTGGCGTTCGGCACTAGACTGCTAGTGTCTCCGATATGAAGTCTGGAGCAC 1320  
-----+-----+-----+-----+-----+-----+  
GTGCCGGGACCGCAAGCCGTGATCTGACGATCACAGGAGGCTATACTTCAGGACCTCGTG  
T A L A F G T R L L V S S D M K S W S T -

1321 TGTCTGGCAGTGATGGGGAAAGCTTTTTCTGAAGCTGCCTTCACCACTGCCTACCTGTT 1380  
-----+-----+-----+-----+-----+-----+  
ACAGGACCGTCACTACCCCTTTGAAAAAGACTTCGACGGAAGTGGTGACGGATGGACAA  
V L A V M G K A F S E A A F T T A Y L F -

1381 CACTTCAGAGTTGTACCCTACGGTGTCTCAGACAGACAGGGATGGGGCTGACTGCACTGGT 1440  
-----+-----+-----+-----+-----+-----+  
GTGAAGTCTCAACATGGGATGCCACGAGTCTGTCTGTCCCTACCCCGACTGACGTGACCA  
T S E L Y P T V L R Q T G M G L T A L V -

1441 GGGCCGGCTGGGGGGCTCTTTGGCCCCACTGGCGGCCTTGCTGGATGGAGTGTGGCTGTC 1500  
-----+-----+-----+-----+-----+-----+  
CCCGGCCGACCCCCCGAGAAACCGGGGTGACCGCCGGAACGACCTACCTCACACCGACAG  
G R L G G S L A P L A A L L D G V W L S -

1501 ACTGCCCAAGCTTACTTATGGGGGGATCGCCCTGCTGGCTGCCGGCACCGCCCTCCTGCT 1560  
-----+-----+-----+-----+-----+-----+  
TGACGGGTTCGAATGAATACCCCCCTAGCGGGACGACCGACGGCCGTGGCGGGAGGACGA  
L P K L T Y G G I A L L A A G T A L L L -

1561 GCCAGAGACGAGGCAGGCACAGCTGCCAGAGACCATCCAGGACGTGGAGAGAAAGAGAGA 1620  
-----+-----+-----+-----+-----+-----+  
CGGTCTCTGCTCCGTCCGTGTCGACGGTCTCTGGTAGGTCCTGCACCTCTCTTTCTCTCT  
P E T R Q A Q L P E T I Q D V E R K R D -

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TGGTGCTAAAGAAAGGACTAGCATATGAGACTTCTGGTACCAATGGGGCTGGTGGGCATG  
-----+-----+-----+-----+-----+-----+-----+  
ACCACGATTTCCTTCCTGATCGTATACTCTGAAGACCATGGTTACCCCGACCACCCGTAC  
  
G A K E R T S I \*  
  
CTGTCCACTGTGTGGTGCTAGGACTGCCAATGCCAGGCCCAAGGGACAAAAAGAACAGAG  
-----+-----+-----+-----+-----+-----+-----+  
GACAGGTGACACACCACGATCCTGACGGTTACGGTCCGGGTTCCCTGTTTTCTTGTCTC  
  
CTTTTTGTTCTCATGGCTGGCCCTGCTACCTCCGAGGCACCCTGCAGGGCAATGCATGTC  
-----+-----+-----+-----+-----+-----+-----+  
GAAAAACAAGAGTACCGACCGGGACGATGGAGGCTCCGTGGGACGTCCCGTTACGTACAG  
  
ATCCCAACCCCCACACTCCCCATCCTCCAACCCACTGGTCTCATGCCCAAAGAAGAGTTG  
-----+-----+-----+-----+-----+-----+-----+  
TAGGGTTGGGGGTGTGAGGGGTAGGAGGTTGGGTGACCAGAGTACGGGTTTCTTCTCAAC  
  
AAGGCATGGGAGCCAACATTTTATTGAAGAAGCCACAGAGGCTGAAATTCAATAAACACA  
-----+-----+-----+-----+-----+-----+-----+  
TTCCGTACCCTCGGTTGTAAAATAACTTCTTCGGTGTCTCCGACTTTAAGTTATTTGTGT  
  
AGTTTTATGAGTAAAAAAAAAAAAAAAAAAAA  
-----+-----+-----+  
TCAAATACTCATTTTTTTTTTTTTTTTTTTT

1680

1740

1800

1860

1920

1980

FIG. 3D

|     |   |     |
|-----|---|-----|
| 1   | GCAGGGACCTCAACTACACTGATCACCAGCCCCATCGGATCCAGACCCGGCCACCAGTGC<br>-----+-----+-----+-----+-----+-----+-----+<br>CGTCCCTGGAGTTGATGTGACTAGTGGTCGGGGTAGCCTAGGTCTGGGCCGGTGGTCACG  | 60  |
| 61  | CATGACCTTCTCGGAGATCCTGGACCGTGTGGGAAGCATGGGCCATTTCCAGTTCCTGCA<br>-----+-----+-----+-----+-----+-----+-----+<br>GTACTGGAAGAGCCTCTAGGACCTGGCACACCCTTCGTACCCGGTAAAGGTCAAGGACGT  | 120 |
|     | M T F S E I L D R V G S M G H F Q F L H -   |     |
| 121 | TGTAGCCATACTGGGCCTCCCGATCCTCAACATGGCCAACCACAACCTGCTGCAGATCTT<br>-----+-----+-----+-----+-----+-----+-----+<br>ACATCGGTATGACCCGGAGGGCTAGGAGTTGTACCGGTTGGTGTGGACGACGTCTAGAA   | 180 |
| 181 | CACAGCCGCCACCCCTGTCCACCACTGTGCGCCGCCCCACAATGCCTCCACAGGGCCTTG<br>-----+-----+-----+-----+-----+-----+-----+<br>GTGTCGGCGGTGGGGACAGGTGGTGACAGCGGGCGGGGTGTTACGGAGGTGTCCCGGAAC  | 240 |
| 241 | GGTGCTCCCCATGGGCCCAAATGGGAAGCCTGAGAGGTGCCTCCGTTTTGTACATCCGCC<br>-----+-----+-----+-----+-----+-----+-----+<br>CCACGAGGGGTACCCGGGTTTACCCTTCGGACTCTCCACGGAGGCCAAAACATGTAGGCGG | 300 |
| 301 | CAATGCCAGCCTGCCCAATGACACCCAGAGGGCCATGGAGCCATGCCTGGATGGCTGGGT<br>-----+-----+-----+-----+-----+-----+-----+<br>GTTACGGTCGGACGGGTTACTGTGGGTCTCCCGGTACCTCGGTACGGACCTACCGACCCA  | 360 |
| 361 | CTACAACAGCACCAAGGACTCCATTGTGACAGAGTGGGACTTGGTGTGCAACTCCAACAA<br>-----+-----+-----+-----+-----+-----+-----+<br>GATGTTGTGCGTGGTTCCTGAGGTAACACTGTCTCACCTGAACCACACGTTGAGGTTGTT  | 420 |
| 421 | ACTGAAGGAGATGGCCCAGTCTATCTTCATGGCAGGTATACTGATTGGAGGGCTCGTGCT<br>-----+-----+-----+-----+-----+-----+-----+<br>TGACTTCCTCTACCGGGTCAGATAGAAGTACCGTCCATATGACTAACCTCCCGAGCACGA  | 480 |
| 481 | TGGAGACCTGTCTGACAGGTTTGGCCGCAGGCCCATCCTGACCTGCAGCTACCTGCTGCT<br>-----+-----+-----+-----+-----+-----+-----+<br>ACCTCTGGACAGACTGTCCAAACCGGCGTCCGGGTAGGACTGGACGTCGATGGACGACGA  | 540 |
| 541 | GGCAGCCAGCGGCTCCGGTGCAGCCTTCAGCCCCACCTTCCCCATCTACATGGTCTTCCG<br>-----+-----+-----+-----+-----+-----+-----+<br>-----+-----+-----+-----+-----+-----+-----+                    | 600 |

FIG. 4A

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CCGTCGGTCGCCGAGGCCACGTCGGAAGTCGGGGTGAAGGGGTAGATGTACCAGAAGGC  
A A S G S G A A F S P T F P I Y M V F R -  
CTTCCTGTGTGGCTTTGGCATCTCAGGCATTACCCTGAGCACCGTCATCTTGAATGTGGA  
601 -----+-----+-----+-----+-----+-----+-----+ 660  
GAAGGACACACCGAAACCGTAGAGTCCGTAATGGGACTCGTGGCAGTAGAACTTACACCT  
F L C G F G I S G I T L S T V I L N V E -  
ATGGGTGCCTACCCGGATGCGGGCCATCATGTGACAGCACTCGGGTACTGCTACACCTT  
661 -----+-----+-----+-----+-----+-----+-----+ 720  
TACCCACGGATGGGCCTACGCCCCGGTAGTACAGCTGTCGTGAGCCCATGACGATGTGGAA  
W V P T R M R A I M S T A L G Y C Y T F -  
TGGCCAGTTCATTCTGCCCCGGCCTGGCCTACGCCATCCCCCAGTGGCGTTGGCTGCAGTT  
721 -----+-----+-----+-----+-----+-----+-----+ 780  
ACCGGTCAAGTAAGACGGGCCGGACCGGATGCGGTAGGGGGTCACCGCAACCGACGTCAA  
G Q F I L P G L A Y A I P Q W R W L Q L -  
AACTGTGTCCATTCCCTTCTTCGTCTTCTTCCTATCATCCTGGTGGACACCAGAGTCCAT  
781 -----+-----+-----+-----+-----+-----+-----+ 840  
TTGACACAGGTAAGGGAAGAAGCAGAAGAAGGATAGTAGGACCACCTGTGGTCTCAGGTA  
T V S I P F F V F F L S S W W T P E S I -  
ACGCTGGTTGGTCTTGTCTGGAAAGTCCTCGAAGGCCCTGAAGATACTCCGGCGGGTGGC  
841 -----+-----+-----+-----+-----+-----+-----+ 900  
TGCGACCAACCAGAACAGACCTTTCAGGAGCTTCCGGGACTTCTATGAGGCCGCCACCG  
R W L V L S G K S S K A L K I L R R V A -  
TGTCTTCAATGGCAAGAAGGAAGAGGGAGAAAGGCTCAGCTTGGAGGAGCTCAAACCTCAA  
901 -----+-----+-----+-----+-----+-----+-----+ 960  
ACAGAAGTTACCGTTCTTCCTTCTCCCTCTTTCGAGTCGAACCTCCTCGAGTTTGAGTT  
V F N G K K E E G E R L S L E E L K L N -  
CCTGCAGAAGGAGATCTCCTTGGCCAAGGCCAAGTACACCGCAAGTGACCTGTTCCGGAT  
961 -----+-----+-----+-----+-----+-----+-----+ 1020  
GGACGTCTTCCTCTAGAGGAACCGGTTCCGGTTCATGTGGCGTTCACTGGACAAGGCCTA  
L Q K E I S L A K A K Y T A S D L F R I -  
ACCCATGCTGCGCCGCATGACCTTCTGTCTTTCCTGGCCTGGTTTGCTACCGGTTTTGC  
1021 -----+-----+-----+-----+-----+-----+-----+ 1080  
TGGGTACGACGCGCGTACTGGAAGACAGAAAGGGACCGGACCAACGATGGCCAAAACG  
P M L R R M T F C L S L A W F A T G F A -  
CTACTATAGTTTGGCTATGGGTGTGGAAGAATTTGGAGTCAACCTCTACATCCTCCAGAT  
1081 -----+-----+-----+-----+-----+-----+-----+ 1140

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GATGATATCAAACCGATACCCACACCTTCTTAAACCTCAGTTGGAGATGTAGGAGGTCTA  
Y Y S L A M G V E E F G V N L Y I L Q I -  
CATCTTTGGTGGGGTCGATGTCCCAGCCAAGTTCATCACCATCCTCTCCTTAAGCTACCT  
1141 -----+-----+-----+-----+-----+-----+-----+ 1200  
GTAGAAACCACCCCAGCTACAGGGTCGGTTCAAGTAGTGGTAGGAGAGGAATTCGATGGA  
I F G G V D V P A K F I T I L S L S Y L -  
GGGCCGGCATACCACTCAGGCCGCTGCCCTGCTCCTGGCAGGAGGGGCCATCTTGGCTCT  
1201 -----+-----+-----+-----+-----+-----+-----+ 1260  
CCCGGCCGTATGGTGAGTCCGGCGACGGGACGAGGACCGTCCTCCCCGGTAGAACCGAGA  
G R H T T Q A A A L L L A G G A I L A L -  
CACCTTTGTGCCCTTGGACTTGCAGACCGTGAGGACAGTATTGGCTGTGTTTGGGAAGGG  
1261 -----+-----+-----+-----+-----+-----+-----+ 1320  
GTGGAAACACGGGAACCTGAACGTCTGGCACTCCTGTCATAACCGACACAAACCCTTCCC  
T F V P L D L Q T V R T V L A V F G K G -  
ATGCCTATCCAGCTCCTTCAGCTGCCTCTTCCTCTACACAAGTGAATTATACCCACAGT  
1321 -----+-----+-----+-----+-----+-----+-----+ 1380  
TACGGATAGGTCGAGGAAGTCGACGGAGAAGGAGATGTGTTCACTTAATATGGGGTGTCA  
C L S S S F S C L F L Y T S E L Y P T V -  
CATCAGGCAAACAGGTATGGGCGTAAGTAACCTGTGGACCCGCGTGGGAAGCATGGTGTC  
1381 -----+-----+-----+-----+-----+-----+-----+ 1440  
GTAGTCCGTTTGTCCATACCCGCATTCAATTGGACACCTGGGCGCACCTTCGTACCACAG  
I R Q T G M G V S N L W T R V G S M V S -  
CCCGCTGGTGAAAATCACGGGTGAGGTACAGCCCTTCATCCCCAATATCATCTACGGGAT  
1441 -----+-----+-----+-----+-----+-----+-----+ 1500  
GGGCGACCACTTTTAGTGCCCACTCCATGTGCGGAAGTAGGGGTTATAGTAGATGCCCTA  
P L V K I T G E V Q P F I P N I I Y G I -  
CACCGCCCTCCTCGGGGGCAGTGCTGCCCTCTTCCTGCCTGAGACCCTGAATCAGCCCTT  
1501 -----+-----+-----+-----+-----+-----+-----+ 1560  
GTGGCGGGAGGAGCCCCCGTCACGACGGGAGAAGGACGGACTCTGGGACTTAGTCGGGAA  
T A L L G G S A A L F L P E T L N Q P L -  
GCCAGAGACTATCGAAGACCTGGAAAACCTGGTCCCTGCGGGCAAAGAAGCCAAAGCAGGA  
1561 -----+-----+-----+-----+-----+-----+-----+ 1620  
CGGTCTCTGATAGCTTCTGGACCTTTTGACCAGGGACGCCCGTTTCTTCGGTTTCGTCCT  
P E T I E D L E N W S L R A K K P K Q E -  
GCCAGAGGTGGAAAAGGCCTCCCAGAGGATCCCTCTACAGCCTCACGGACCAGGCCTGGG  
1621 -----+-----+-----+-----+-----+-----+-----+ 1680

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CGGTCTCCACCTTTTCCGGAGGGTCTCCTAGGGAGATGTCGGAGTGCCTGGTCCGGACCC  
P E V E K A S Q R I P L O P H G P G L G -

1681 CTCCAGCTGAGGACAACGGAACCCCTTTCCCTGCCCTCCAGAGACTGATCCTAGCCAGG 1740  
-----+-----+-----+-----+-----+-----+  
GAGGTCGACTCCTGTTGCCTTGGGGGAAAGGGACGGGAGGTCTCTGACTAGGATCGGTCC  
S S \*

1741 CACCTTAGGAGTATAGGGAGGCCCATATAGGTCCATCCTCCTAGGATGAAGCCTTCTGA 1800  
-----+-----+-----+-----+-----+-----+  
GTGGAATCCTCATATCCCTCCGGGTATATCCAGGTAGGAGGATCCTACTTCGGAAGACT

1801 GAGCTTGGTGAAGGTGTCTCCATCACCACCACCAGAGCCTCCTGCCCAGCCCTGGCCAGT 1860  
-----+-----+-----+-----+-----+-----+  
CTCGAACCCTTCCACAGAGGTAGTGGTGGTGGTCTCGGAGGACGGGTCTGGGACCGGTCA

1861 TCAAAGGTTCAAGCCATCCCTGCCCTTGTTCTCCCTGCAACCCAAGCCCTGCCATTCTTC 1920  
-----+-----+-----+-----+-----+-----+  
AGTTTCCAAGTTCGGTAGGGACGGGAACAAGAGGGACGTTGGGTTCGGGACGGTAAGAAG

1921 TGTCTAGCCCTTCCCCACTGGCCAACCTTCCCCACTGTCCCGGTCCTCTTCCCCTGAGGT 1980  
-----+-----+-----+-----+-----+-----+  
ACAGATCGGGAAGGGGTGACCGGTTGAAGGGGGTGACAGGGCCAGGAGAAGGGGACTCCA

1981 CCCCTGATATCCCCTGGCTCAGTCCTAACAAGACTGAGTCTTAACAAGATGAGAAGTCCT 2040  
-----+-----+-----+-----+-----+-----+  
GGGGACTATAGGGGACCGAGTCAGGATTGTTCTGACTCAGAATTGTTCTACTCTTCAGGA

2041 CCCCTTCTTGCCTCCCACACTTTTCTTIGATGGGAGGTTTCAATAAACAGCGATAAGAAC 2100  
-----+-----+-----+-----+-----+-----+  
GGGGAAGAACGGAGGGTGTGAAAAGAACTACCCTCCAAAGTTATTTGTCGCTATTCTTG

2101 TCTAAAAAAAAAAAAAAAAAAAAA 2121  
-----+-----+-----  
AGATTTTTTTTTTTTTTTTTTT

*FIG. 4D*

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1 CAAATTATTTCTTACGTGACTTTAGAGAAAACGGCTACCTATCTGACCCCAAACGACTT 60  
-----+-----+-----+-----+-----+-----+  
GTTTAATAAAGAATGCACTGAAATCTCTTTTGCCGATGGATAGACTGGGGTTTTGCTGAA  
61 GAGGAAACTGTTTCCACGGTCCTGCTGCAGGGGGGAAGCACAGTCGTCAAGAAGAGAGTG 120  
-----+-----+-----+-----+-----+-----+  
CTCCTTTGACAAAGGTGCCAGGACGACGTCCCCCTTCGTGTCAGCAGTTCTTCTCTCAC  
121 GGGTCAGGATCAAAACACATTTAGTGTGACTTAGGGAAAGAAAACATTTTCCCTCTTTGA 180  
-----+-----+-----+-----+-----+-----+  
CCCAGTCCTAGTTTTGTGTAAATCACACTGAATCCCTTTCTTTTGTAAGGGAGAACT  
181 ACCTCTCTGGATACAGTCATTTTGCCTCTACTTGAGGATCAACTGTTCAACCTCAATGGC 240  
-----+-----+-----+-----+-----+-----+  
TGGAGAGACCTATGTCAGTAAAACGGAGATGAACTCCTAGTTGACAAGTTGGAGTTACCG  
M A -  
241 CTTTCAGGACCTCCTGGGTACGCTGGTGACCTGTGGAGATTCCAGATCCTTCAGACTGT 300  
-----+-----+-----+-----+-----+-----+  
GAAAGTCCTGGAGGACCCAGTGCGACCACTGGACACCTCTAAGGTCTAGGAAGTCTGACA  
F Q D L L G H A G D L W R F Q I L Q T V -  
301 TTTTCTCTCAATCTTTGCTGTTGCTACATACCTTCATTTTATGCTGGAGAACTTCACTGC 360  
-----+-----+-----+-----+-----+-----+  
AAAAGAGAGTTAGAAACGACAACGATGTATGGAAGTAAATACGACCTCTTGAAGTGACG  
F L S I F A V A T Y L H F M L E N F T A -  
361 ATTCATACCTGGCCATCGCTGCTGGGTCCACATCCTGGACAATGACACTGTCTCTGACAA 420  
-----+-----+-----+-----+-----+-----+  
TAAGTATGGACCGGTAGCGACGACCCAGGTGTAGGACCTGTTACTGTGACAGAGACTGTT  
F I P G H R C W V H I L D N D T V S D N -  
421 TGACACTGGGGCCCTCAGCCAAGATGCACTCTTGAGAATCTCCATCCCACTGGACTCAAA 480  
-----+-----+-----+-----+-----+-----+  
ACTGTGACCCCGGGAGTCGGTTCTACGTGAGAACTCTTAGAGGTAGGGTGACCTGAGTTT  
D T G A L S Q D A L L R I S I P L D S N -  
481 CATGAGGCCAGAGAAGTGTCGTCGCTTTGTTTCATCCTCAGTGGCAGCTCCTTCACCTGAA 540  
-----+-----+-----+-----+-----+-----+  
GTACTCCGGTCTCTTCACAGCAGCGAAACAAGTAGGAGTCACCGTCGAGGAAGTGGACTT  
M R P E K C R R F V H P Q W Q L L H L N -  
541 TGGGACCTTCCCAACACAAGTGACGCAGACATGGAGCCCTGTGTGGATGGCTGGGTGTA 600  
-----+-----+-----+-----+-----+-----+  
ACCCTGGAAGGGGTTGTGTTCACTGCGTCTGTACCTCGGGACACACCTACCGACCCACAT  
G T F P N T S D A D M E P C V D G W V Y -

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601 TGACAGAATCTCCTTCTCATCCACCATCGGTGACCTGAAGTGGGATCTGGTATGTGACTC 660  
-----+-----+-----+-----+-----+-----+  
ACTGTCTTAGAGGAAGAGTAGGTGGTAGCCACTGGACTTCACCCTAGACCATACTGAG  
D R I S F S S T I G D L K W D L V C D S -  
661 TCAATCACTGACTTCAGTGGCTAAATTGTATTCATGGCTGGAATGATGTTGGGAGGCAT 720  
-----+-----+-----+-----+-----+-----+  
AGTTAGTGACTGAAGTCACCGATTAAACATAAGTACCGACCTTACTACAACCCTCCGTA  
Q S L T S V A K F V F M A G M M L G G I -  
721 CCAGGTTGCCATTGTTGGCACTTGTGCAGCGTTGGCTCCCACTTTCCTCATTACTGCTC 780  
-----+-----+-----+-----+-----+-----+  
GGTCCAACGGTAACAACCGTGAACACGTCGCAACCGAGGGTGAAAGGAGTAAATGACGAG  
L G V H L S D R F G R S F V L R W C Y L -  
781 CCAGGTTGCCATTGTTGGCACTTGTGCAGCGTTGGCTCCCACTTTCCTCATTACTGCTC 840  
-----+-----+-----+-----+-----+-----+  
GGTCCAACGGTAACAACCGTGAACACGTCGCAACCGAGGGTGAAAGGAGTAAATGACGAG  
Q V A I V G T C A A L A P T F L I Y C S -  
841 AGTACGCTTCTTGTCTGGGATTGCTGCAATGAGCTTCATAACAAATACTATTATGTTAAT 900  
-----+-----+-----+-----+-----+-----+  
TCATGCGAAGAACAGACCCTAACGACGTTACTCGAAGTATTGTTTATGATAATACAATTA  
V R F L S G I A A M S F I T N T I M L I -  
901 AGCCGAGTGGGCAACACACAGATTCCAGGCCATGGGAATTACATTGGGAATGTGCCCTTC 960  
-----+-----+-----+-----+-----+-----+  
TCGGCTCACCCGTTGTGTGTCTAAGGTCCGGTACCCTTAATGTAACCCTTACACGGGAAG  
A E W A T H R F Q A M G I T L G M C P S -  
961 TGGTATTGCATTTATGACCCTGGCAGGCCTGGCTTTTGCCATTCGAGACTGGCATATCCT 1020  
-----+-----+-----+-----+-----+-----+  
ACCATAACGTAAATACTGGGACCGTCCGGACCGAAAACGGTAAGCTCTGACCGTATAGGA  
G I A F M T L A G L A F A I R D W H I L -  
1021 CCAGCTGGTGGTGTCTGTACCATACTTTGTGATCTTCTGACCTCAAGTTGGCTGCTAGA 1080  
-----+-----+-----+-----+-----+-----+  
GGTCGACCACCACAGACATGGTATGAAACACTAGAAAGACTGGAGTTCAACCGACGATCT  
Q L V V S V P Y F V I F L T S S W L L E -  
1081 GTCTGCTCGGTGGCTCATTATCAACAATAAACCAGAGGAAGGCTTAAAGGAACCTTAGAAA 1140  
-----+-----+-----+-----+-----+-----+  
CAGACGAGCCACCGAGTAATAGTTGTTATTTGGTCTCCTTCCGAATTCCTTGAATCTTT  
S A R W L I I N N K P E E G L K E L R K -

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1141 AGCTGCACACAGGAGTGGAATGAAGAATGCCAGAGACACCCTAACCCCTGGAGATTTTGAA 1200  
-----+-----+-----+-----+-----+-----+  
TCGACGTGTGTCCTCACCTTACTTCTTACGGTCTCTGTGGGATTGGGACCTCTAAACTT  
A A H R S G M K N A R D T L T L E I L K -  
1201 ATCCACCATGAAAAAAGAACTGGAGGCAGCACAAAAAAAACCTTCTCTGTGTGAAAT 1260  
-----+-----+-----+-----+-----+-----+  
TAGGTGGTACTTTTTTCTTGACCTCCGTGCTGTTTTTTTTTTTGAAGAGACACACTTA  
S T M K K E L E A A Q K K K P S L C E M -  
1261 GCTCCACATGCCCAACATATGTAAAAGGATCTCCCTCCTGTCCTTTACGAGATTTGCAA 1320  
-----+-----+-----+-----+-----+-----+  
CGAGGTGTACGGGTTGTATACATTTTCCTAGAGGGAGGACAGGAAATGCTCTAAACGTTT  
L H M P N I C K R I S L L S F T R F A N -  
1321 CTTTATGGCCTATTTTGGCCTTAATCTCCATGTCCAGCATCTGGGGAACAATGTTTTCT 1380  
-----+-----+-----+-----+-----+-----+  
GAAATACCGGATAAAACCGGAATTAGAGGTACAGGTCGTAGACCCCTTGTTACAAAAGGA  
F M A Y F G L N L H V Q H L G N N V F L -  
1381 GTTGCAGACTCTCTTTGGTGCAGTCATCCTCCTGGCCAACTGTGTTGCACCTTGGGCACT 1440  
-----+-----+-----+-----+-----+-----+  
CAACGTCTGAGAGAAACCACGTCAGTAGGAGGACCGGTTGACACAACGTGGAACCCGTGA  
L Q T L F G A V I L L A N C V A P W A L -  
1441 GAAATACATGAACCGTCGAGCAAGCCAGATGCTTCTCATGTTCTACTGGCAATCTGCCT 1500  
-----+-----+-----+-----+-----+-----+  
CTTTATGTACTTGGCAGCTCGTTCCGTCTACGAAGAGTACAAGGATGACCGTTAGACGGA  
K Y M N R R A S Q M L L M F L L A I C L -  
1501 TCTGGCCATCATATTTGTGCCACAAGAAATGCAGACGCTGCGTGAGGTTTTGGCAACACT 1560  
-----+-----+-----+-----+-----+-----+  
AGACCGGTAGTATAAACACGGTGTCTTTACGTCTGCGACGCACTCCAAAACCGTTGTGA  
L A I I F V P Q E M Q T L R E V L A T L -  
1561 GGGCTTAGGAGCGTCTGCTCTTGCCAATACCCTTGCTTTTGCCCATGGAAATGAAGTAAT 1620  
-----+-----+-----+-----+-----+-----+  
CCCGAATCCTCGCAGACGAGAACGGTTATGGGAACGAAAACGGGTACCTTTACTTCATTA  
G L G A S A L A N T L A F A H G N E V I -  
1621 TCCCACCATAATCAGGGCAAGAGCTATGGGGATCAATGCAACCTTTGCTAATATAGCAGG 1680  
-----+-----+-----+-----+-----+-----+  
AGGGTGGTATTAGTCCCGTTCTCGATACCCCTAGTTACGTTGGAAACGATTATATCGTCC  
P T I I R A R A M G I N A T F A N I A G -

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1681 AGCCCTGGCTCCCCTCATGATGATCCTAAGTGTGTATTCTCCACCCCTGCCCTGGATCAT  
-----+-----+-----+-----+-----+-----+ 1740  
TCGGGACCGAGGGGAGTACTACTAGGATTCACACATAAGAGGTGGGGACGGGACCTAGTA  
A L A P L M M I L S V Y S P P L P W I I -  
1741 CTATGGAGTCTTCCCCTTCATCTCTGGCTTTGCTTTCCTCCTTCCTGAAACCAGGAA  
-----+-----+-----+-----+-----+ 1800  
GATACCTCAGAAGGGGAAGTAGAGACCGAAACGAAAGGAGGAGGAAGGACTTTGGTCCTT  
Y G V F P F I S G F A F L L L P E T R N -  
1801 CAAGCCTCTGTTTGACACCATCCAGGATGAGAAAAATGAGAGAAAAGACCCCAGAGAACC  
-----+-----+-----+-----+-----+ 1860  
GTTTCGGAGACAAACTGTGGTAGGTCTACTCTTTTTACTCTCTTTTCTGGGGTCTCTTGG  
K P L F D T I Q D E K N E R K D P R E P -  
1861 AAAGCAAGAGGATCCGAGAGTGGAAGTGACGCAGTTTTAAGGAATTCCAGGAGCTGACTG  
-----+-----+-----+-----+-----+ 1920  
TTTCGTTCTCCTAGGCTCTCACCTTCACTGCGTCAAAATTCCTTAAGGTCCTCGACTGAC  
K Q E D P R V E V T Q F \*  
1921 CCGATCAATGAGCCAGATGAAGGGAACAATCAGGACTATTCCTAGACACTAGCAAAA  
-----+-----+-----+-----+-----+ 1977  
GGCTAGTTACTCGGTCTACTTCCCTTGTTAGTCCTGATAAGGATCTGTGATCGTTTT

*FIG. 5D*

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CTCCTGATAGCAAAAGAACTGAGGAAGCTCTTTCCACTACGGCTGTATTGCACTGGTGAG  
-----+-----+-----+-----+-----+-----+-----+  
1 GAGGACTATCGTTTTCTTGACTCCTTCGAGAAAGGTGATGCCGACATAACGTGACCACTC 60  
TCCGGGCCCCATGGATGAGAAATTGATGCGAGGATCAATACAAGCTTAATTTGAATTAATA  
-----+-----+-----+-----+-----+-----+-----+  
61 AGGCCCCGGGTACCTACTCTTTAACTACGCTCCTAGTTATGTTTCAATTAACTTAATTAT 120  
AAAGGAAATATTTTCTCCCTTTGAACTTATCTCCGTAAAGCCATTGTGCCTCCTCTTGGG  
-----+-----+-----+-----+-----+-----+-----+  
121 TTTCCTTTATAAAAGAGGGAACTTGAATAGAGGCATTTCCGTAAACACGGAGGAGAACCC 180  
GGTCACGTGTTTACAATCAATGGCCTTTGAGGAGCTCTTGAGTCAAGTTGGAGGCCTTGG  
-----+-----+-----+-----+-----+-----+-----+  
181 CCAGTGCACAAGTGTTAGTTACCGGAACTCCTCGAGAACTCAGTTCAACCTCCGGAACC 240  
M A F E E L L S Q V G G L G -  
GAGATTTTCAAGTCTTCATCTGGTTTTTATTCTTCCCTCTCTCATGTTATTAATCCCTCA  
-----+-----+-----+-----+-----+-----+-----+  
241 CTCTAAAGTCTACGAAGTAGACCAAAAATAAGAAGGGAGAGAGTACAATAATTAGGGAGT 300  
R F Q M L H L V F I L P S L M L L I P H -  
TATACTGCTAGAGAACTTTGCTGCAGCCATTCTGGTTCATCGTTGCTGGGTCCACATGCT  
-----+-----+-----+-----+-----+-----+-----+  
301 ATATGACGATCTCTTGAAACGACGTCGGTAAGGACCAGTAGCAACGACCCAGGTGTACGA 360  
I L L E N F A A A I P G H R C W V H M L -  
GGACAATAATACTGGATCTGGTAATGAACTGGAATCCTCAGTGAAGATGCCCTCTTGAG  
-----+-----+-----+-----+-----+-----+-----+  
361 CCTGTTATTATGACCTAGACCATTACTTTGACCTTAGGAGTCACTTCTACGGGAGAACTC 420  
D N N T G S G N E T G I L S E D A L L R -  
AATCTCTATCCCACTAGACTCAAATCTGAGGCCAGAGAAGTGTCGTCGCTTTGTCCATCC  
-----+-----+-----+-----+-----+-----+-----+  
421 TTAGAGATAGGGTGATCTGAGTTTAGACTCCGGTCTCTTCACAGCAGCGAAACAGGTAGG 480  
I S I P L D S N L R P E K C R R F V H P -  
CCAGTGGCAGCTTCTTCACCTGAATGGGACTATCCACAGCACAAGTGAGGCAGACACAGA  
-----+-----+-----+-----+-----+-----+-----+  
481 GGTCACCGTCGAAGAAGTGGAATACCCTGATAGGTGTCGTGTTCACTCCGTCTGTGTCT 540  
Q W Q L L H L N G T I H S T S E A D T E -  
ACCCTGTGTGGATGGCTGGGTATATGATCAAAGCTACTTCCCTTCGACCATTGTGACTAA  
-----+-----+-----+-----+-----+-----+-----+  
541 TGGGACACACCTACCGACCCATATACTAGTTTCGATGAAGGGAAGCTGGTAACAATGATT 600  
P C V D G W V Y D O S Y F P S T I V T K -

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601 GTGGGACCTGGTATGTGATTATCAGTCACTGAAATCAGTGGTTCAATTCCTACTTCTGAC 660  
-----+-----+-----+-----+-----+-----+  
CACCTGGACCATACTAATAGTCAGTGACTTTAGTCACCAAGTTAAGGATGAAGACTG  
W D L V C D Y Q S L K S V V Q F L L L T -  
661 TGGAAATGCTGGTGGGAGGCATCATAGGTGGCCATGTCTCAGACAGGTTTGGGCGAAGATT 720  
-----+-----+-----+-----+-----+-----+  
ACCTTACGACCACCCTCCGTAGTATCCACCGGTACAGAGTCTGTCCAAACCCGCTTCTAA  
G M L V G G I I G G H V S D R F G R R F -  
721 TATTCTCAGATGGTGTGTTTGCTCCAGCTTGCCATTACTGACACCTGCGCTGCCTTCGCTCC 780  
-----+-----+-----+-----+-----+-----+  
ATAAGAGTCTACCACAAACGAGGTGCGAACGGTAATGACTGTGGACGACGCGGAAGCGAGG  
I L R W C L L Q L A I T D T C A A F A P -  
781 CACCTTCCCTGTTTACTGTGTACTACGCTTCTTGGCAGGTTTTTCTTCCATGATCATTAT 840  
-----+-----+-----+-----+-----+-----+  
GTGGAAGGGACAAATGACACATGATGCGAAGAACCGTCCAAAAAGAAGGTACTAGTAATA  
T F P V Y C V L R F L A G F S S M I I I -  
841 ATCAAATAATTCTTTGCCCATTACTGAGTGGATAAGGCCCAACTCTAAAGCCCTGGTAGT 900  
-----+-----+-----+-----+-----+-----+  
TAGTTTATTAAGAAACGGGTAATGACTCACCTATTCCGGGTTGAGATTTCTGGGACCATCA  
S N N S L P I T E W I R P N S K A L V V -  
901 AATATTGTCATCTGGTGGCCTTAGTATTGGACAGATAATCCTGGGAGGCTTGGCTTATGT 960  
-----+-----+-----+-----+-----+-----+  
TTATAACAGTAGACCACGGGAATCATAACCTGTCTATTAGGACCCTCCGAACCGAATACA  
I L S S G A L S I G Q I I L G G L A Y V -  
961 CTTCCGAGACTGGCAAACCCTGCACGTGGTGGCGTCAGTACCTTTCCTTGGCCTCCTTCT 1020  
-----+-----+-----+-----+-----+-----+  
GAAGGCTCTGACCGTTTGGGACGTGCACCACCGCAGTCATGGAAAGGAACCGGAGGAAGA  
F R D W Q T L H V V A S V P F L G L L L -  
1021 CCTTCAAAGGTGGCTGGTGGGAATCTGCTCGGTGGTTGATAATCACCAATAAACTAGATGA 1080  
-----+-----+-----+-----+-----+-----+  
GGAAGTTTCCACCGACCACCTTAGACGAGCCACCAACTATTAGTGGTTATTTGATCTACT  
L Q R W L V E S A R W L I I T N K L D E -  
1081 GGGCTTAAAGGCACTTAGAAAAGTTGCACGCACAAATGGAATAAAGAATGCTGAAGAAAC 1140  
-----+-----+-----+-----+-----+-----+  
CCCGAATTTCCGTGAATCTTTTCAACGTGCGTGTTTACCTTATTTCTTACGACTTCTTTG  
G L K A L R K V A R T N G I K N A E E T -

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1141 CCTGAACATAGAGGTTGTAAGATCCACCATGCAGGAGGAGCTGGATGCAGCACAGACCAA  
-----+-----+-----+-----+-----+-----+-----+ 1200  
GGACTTGTATCTCCAACATTCTAGGTGGTACGTCCTCCTCGACCTACGTCGTGTCTGGTT  
L N I E V V R S T M Q E E L D A A Q T K -  
1201 AACTACTGTGTGTGACTTGTTCGCAACCCCAGTATGCGTAAAAGGATCTGTATCCTGGT  
-----+-----+-----+-----+-----+-----+ 1260  
TTGATGACACACACTGAACAAGGCGTTGGGGTCATACGCATTTTCCTAGACATAGGACCA  
T T V C D L F R N P S M R K R I C I L V -  
1261 ATTTTGTGAGATTGCAAACACAATACCTTTTATGGTACCATGGTCAATCTTCAGCATGT  
-----+-----+-----+-----+-----+-----+ 1320  
TAAAACTCTAAACGTTTGTGTTATGGAAAAATACCATGGTACCAGTTAGAAGTCGTACA  
F L R F A N T I P F Y G T M V N L Q H V -  
1321 GGGGAGCAACATTTTCCTGTTGCAGGTACTTTATGGAGCTGTCGCTCTCATAGTTTCGATG  
-----+-----+-----+-----+-----+-----+ 1380  
CCCCTCGTTGTAAAAGGACAACGTCCATGAAATACCTCGACAGCGAGAGTATCAAGCTAC  
G S N I F L L Q V L Y G A V A L I V R C -  
1381 TCTTGCTCTTTTGACACTAAATCATATGGGCCGTGCAATAAGCCAGATATTGTTTCATGTT  
-----+-----+-----+-----+-----+-----+ 1440  
AGAACGAGAAAACGTGTGATTTAGTATACCCGGCAGCTTATTCGGTCTATAACAAGTACAA  
L A L L T L N H M G R R I S Q I L F M F -  
1441 CCTGGTGGGCCTTTCCATTTTGGCCAACACGTTTGTGCCCAAAGAAATGCAGACCCTGCG  
-----+-----+-----+-----+-----+-----+ 1500  
GGACCACCCGGAAAGGTAAAACCGGTTGTGCAAACACGGGTTTCTTTACGTCTGGGACGC  
L V G L S I L A N T F V P K E M Q T L R -  
1501 TGTGGCTTTGGCATGTCTGGGAATCGGCTGTTCTGCTGCTACTTTTTCCAGTGTTGCTGT  
-----+-----+-----+-----+-----+-----+ 1560  
ACACCGAAACCGTACAGACCCTTAGCCGACAAGACGACGATGAAAAAGGTCACAACGACA  
V A L A C L G I G C S A A T F S S V A V -  
1561 TCACTTCATTGAACTCATCCCCACTGTTCTCAGGGCAAGAGCTTCAGGAATAGATTTAAC  
-----+-----+-----+-----+-----+-----+ 1620  
AGTGAAGTAACTTGAGTAGGGGTGACAAGAGTCCCGTTCTCGAAGTCCTTATCTAAATTG  
H F I E L I P T V L R A R A S G I D L T -  
1621 GGCTAGTAGGATTGGAGCAGCACTGGCTCCCCTCTTGATGACCTTAACGGTATTTTTTAC  
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CCGATCATCCTAACCTCGTCGTGACCGAGGGGAGAACTACTGGAATTGCCATAAAAAATG  
A S R I G A A L A P L L M T L T V F F T -

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1681 CACTTTGCCATGGATCATTTATGGAATCTTCCCATCATTTGGTGGCCTTATTGTCTTCCT  
-----+-----+-----+-----+-----+-----+-----+ 1740  
GTGAAACGGTACCTAGTAAATACCTTAGAAGGGGTAGTAACCACCGGAATAACAGAAGGA  
T L P W I I Y G I F P I I G G L I V F L -  
1741 CCTACCAGAAACCAAGAATCTGCCTTTGCCTGACACCATCAAGGATGTGGAAAATCAAAA  
-----+-----+-----+-----+-----+-----+-----+ 1800  
GGATGGTCTTTGGTTCTTAGACGGAAACGGACTGTGGTAGTTCCTACACCTTTTAGTTTT  
L P E T K N L P L P D T I K D V E N Q K -  
1801 AAAAAATCTCAAGGAAAAGGCATAAAAAATGATTGCTACACAAAAGTGACCAAATTTTAAG  
-----+-----+-----+-----+-----+-----+-----+ 1860  
TTTTTTAGAGTTCCTTTTCCGTATTTTACTAACGATGTGTTTTCACTGGTTTAAATTC  
K N L K E K A \*  
1861 AAGCCTTCATGAGCTGATTGGTGGGGAAATTCAGAAAAAAAAAATACAGGAAAAGAACACA  
-----+-----+-----+-----+-----+-----+-----+ 1920  
TTCGGAAGTACTCGACTAACCACCCCTTTAAGTCTTTTTTTTTATGTCCTTTTCTTGTGT  
1921 CCAGAAGGGTTTTTTTCCCTACAACCAGCAAGAACATATATTAGATACATGAATCTCAAT  
-----+-----+-----+-----+-----+-----+-----+ 1980  
GGTCTTCCCAAAAAAAGGGATGTTGGTCGTTCTTGTATATAATCTATGTACTTAGAGTTA  
1981 TATAATTATGGCATTAAATTTGCATTTTATTTCAAATTAACCTTGTTGGGGACATGTAATCT  
-----+-----+-----+-----+-----+-----+-----+ 2040  
ATATTAATACCGTAATTAAACGTAAAATAAAGTTTAAATTGAACACCCCTGTACATTAGA  
2041 CTTGAGCAATCTGATATTTTTGGGAAGTCCTTTAAAAAGTTACAAATTTATCAATAAATT  
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GAACTCGTTAGACTATAAAAACCTTCAGGAAATTTTCAATGTTTAAATAGTTATTTAA  
2101 ACTAGTAGATAAGATGATTCAGAAACAAAGGAAAATCACAGAATTAGGATGTGGCTGGCT  
-----+-----+-----+-----+-----+-----+-----+ 2160  
TGATCATCTATTCTACTAAGTCTTTGTTTCCTTTTAGTGTCTTAATCCTACACCGACCGA  
2161 TGGTGTATGAAGCACCATGTGATGAATTCATAAAGTTGCAAAAGTCAAAACAATACTGTA  
-----+-----+-----+-----+-----+-----+-----+ 2220  
ACCACATACTTCGTGGTACACTACTTAAGTATTTCAACGTTTTAGTTTTGTTATGACAT  
2221 CATGCAACCAGAAATCAAATTAATCCAGAAATAGAGACCTATATAAATGCATTTAATAC  
-----+-----+-----+-----+-----+-----+-----+ 2280  
GTACGTTGGTCTTTAGTTTAATTTAGGTCTTTATCTCTGGATATATTTACGTAAATTATG  
2281 ATGATACTTTTGACATATTAAGCCATTGGAAAACGGAAGGATTAGATACTTAAATAACAT  
-----+-----+-----+-----+-----+-----+-----+ 2340  
TACTATGAAAACGTGATAATTCGGTAACCTTTGCCTTCCTAATCTATGAATTTATTGTA  
2341 TGCTATCTCTTTGTAAATACAGTCACTAAATGATGTTAGTTACTTTTCCATGGTGGAATT  
-----+-----+-----+-----+-----+-----+-----+ 2400

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2401 TTAATTACTTTTTCTTTGTAATTTTTCTCTGTATATTTTAAACAAATAGCTGGTATAG 2460  
-----+-----+-----+-----+-----+-----+  
AATTAATGAAAAAGAAACATTA AAAAGAGAGACATATAAAATTTGTTTATCGACCATATC

2461 TTTACAATATTATAAAGATATTGTTCAAATTGAAGGGCAAAGGCCAGGTTTCAGCAATTTT 2520  
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AAATGTTATAATATTTCTATAACAAGTTTAACTTCCCGTTTCCGGTCCAAGTCGTTAAAA

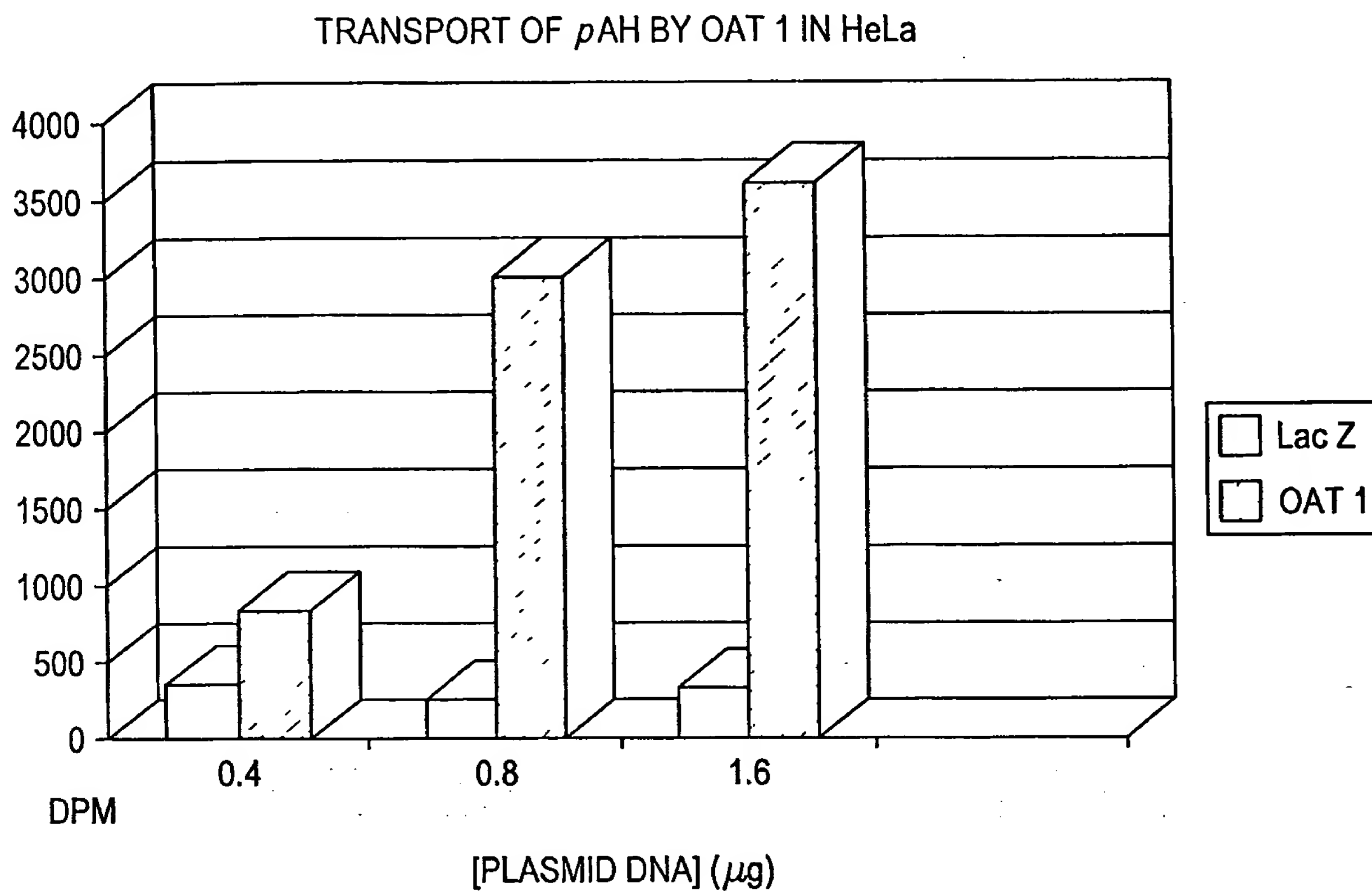
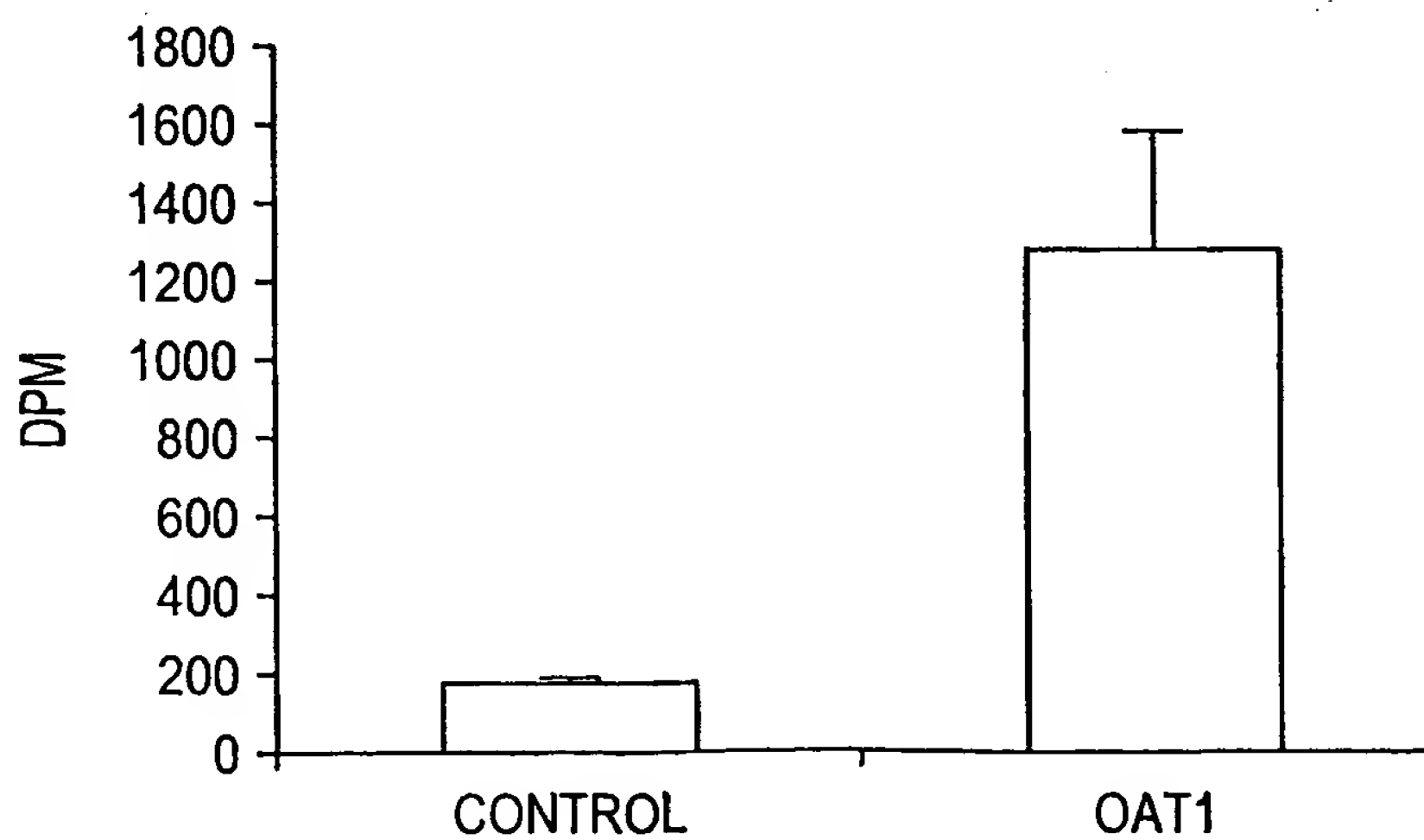
2521 CAAACTGTATGTACATTTAATAAAAATAACTATAAATTAAAAAATTATATTTCAAATGATG 2580  
-----+-----+-----+-----+-----+-----+  
GTTTGACATACATGTAAATTATTTTATTGATATTTAATTTTTTAATATAAAGTTTACTAC

2581 TGACTAATAAATGAAAGTACATATAGTAGTAAAGTAATTTTCAGGCAAACCTATATAACCA 2640  
-----+-----+-----+-----+-----+-----+  
ACTGATTATTTACTTTTCATGTATATCATCATTTCATTAAAGTCCGTTTGGATATATTGGT

2641 AAATATAAACTTTTCATTTTAAACAGCAAAAAAAAAAAAAAAAAAAAAA 2684  
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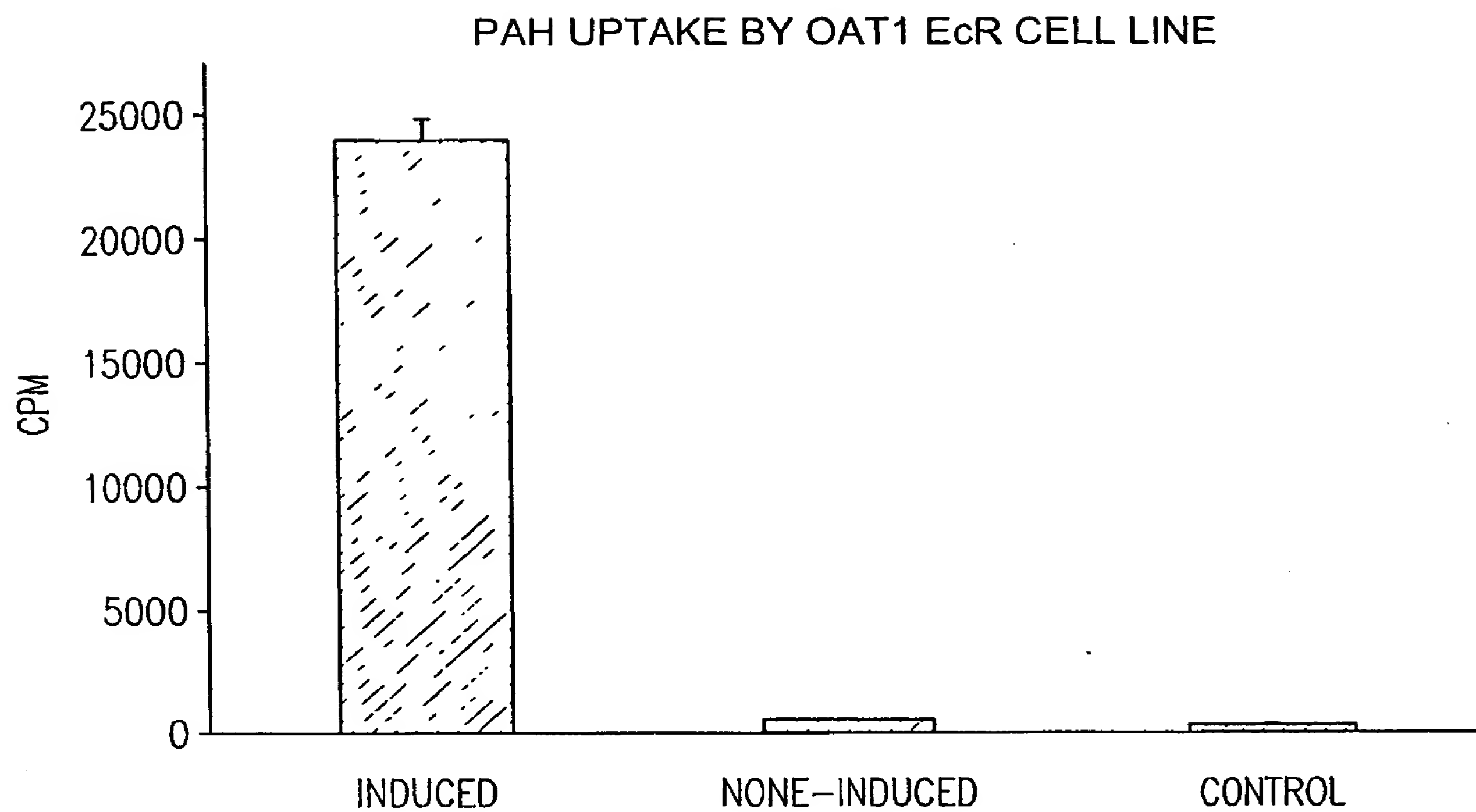
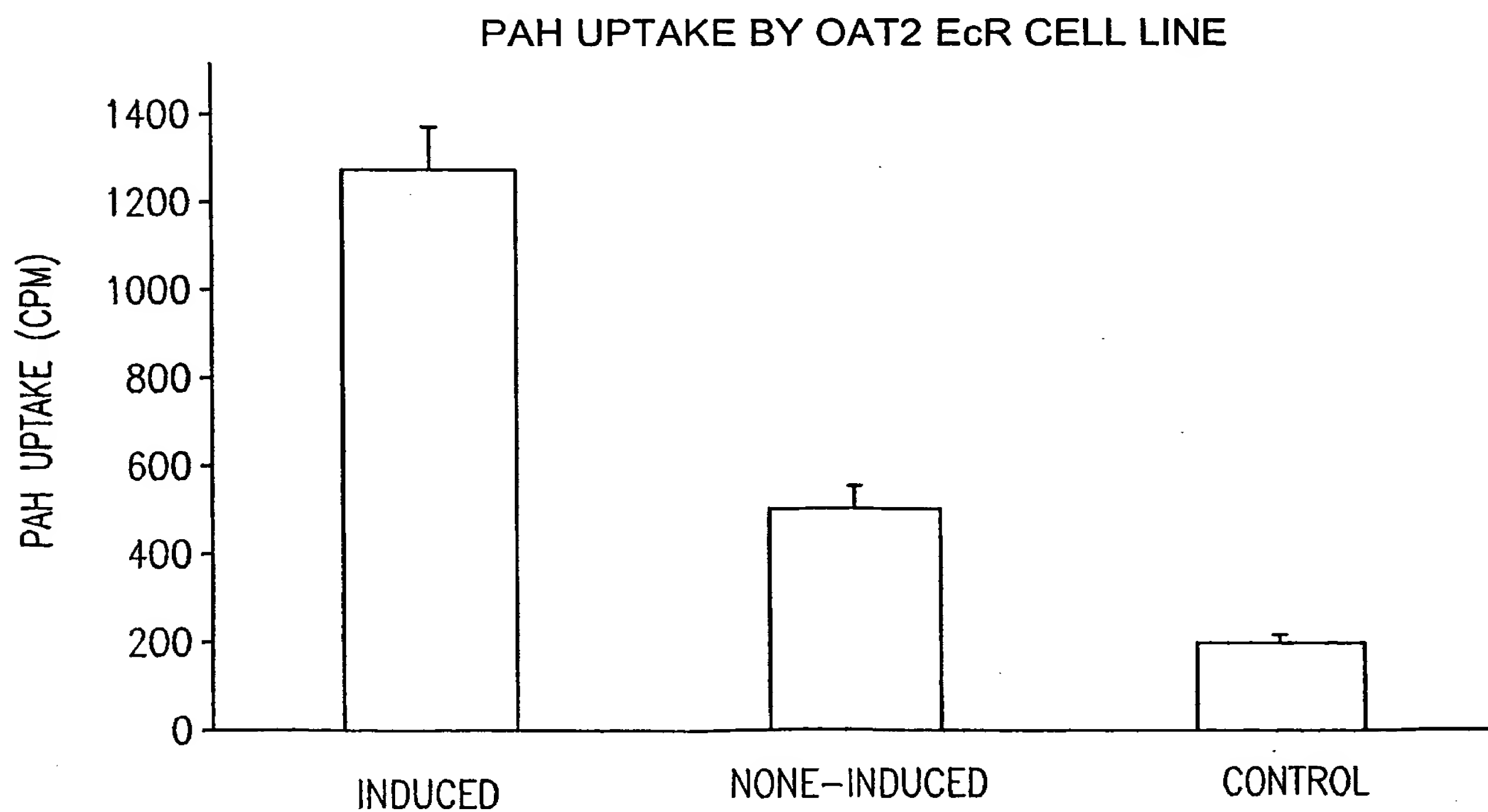
*FIG. 6E*

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*FIG. 7*

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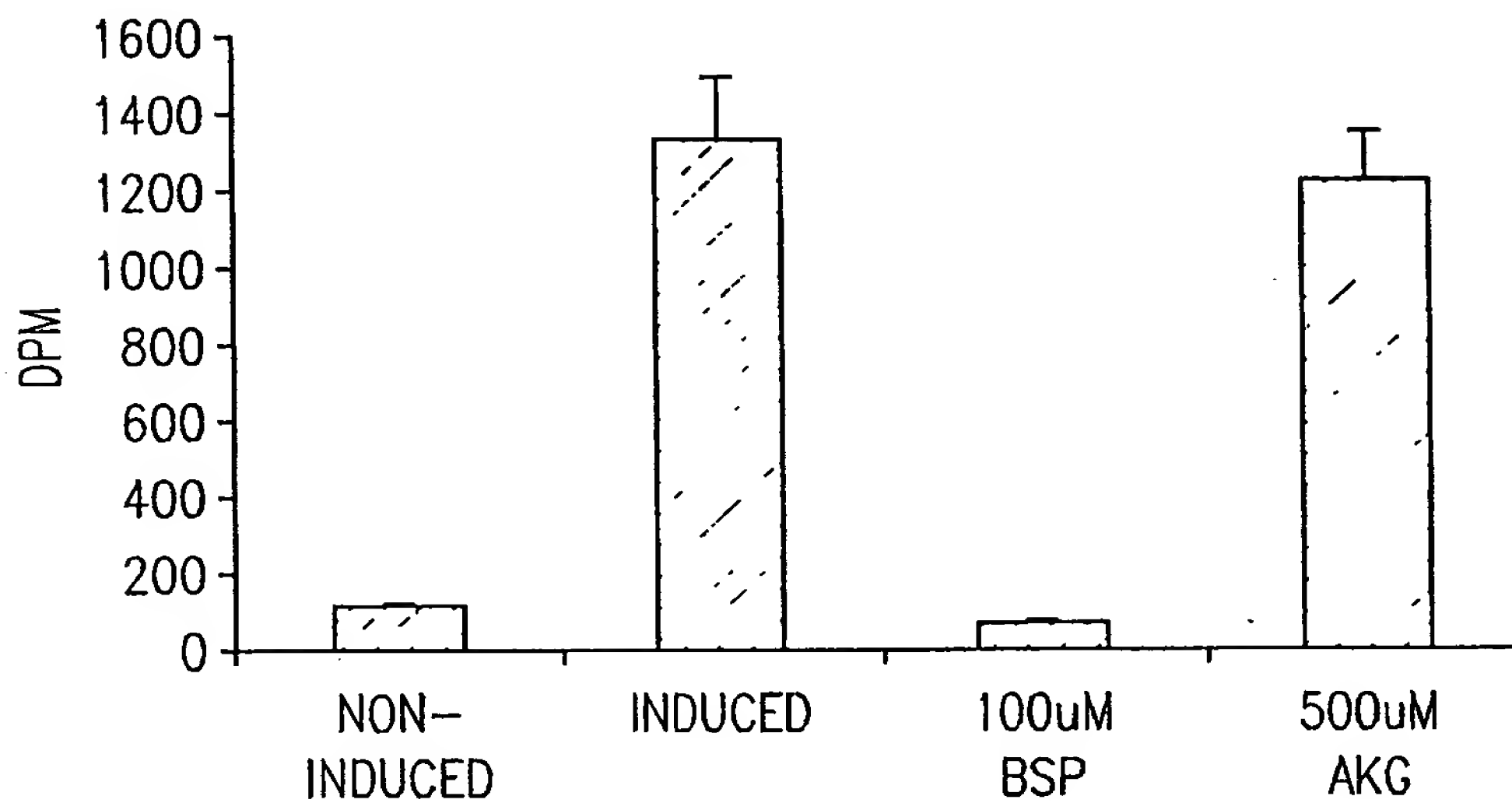
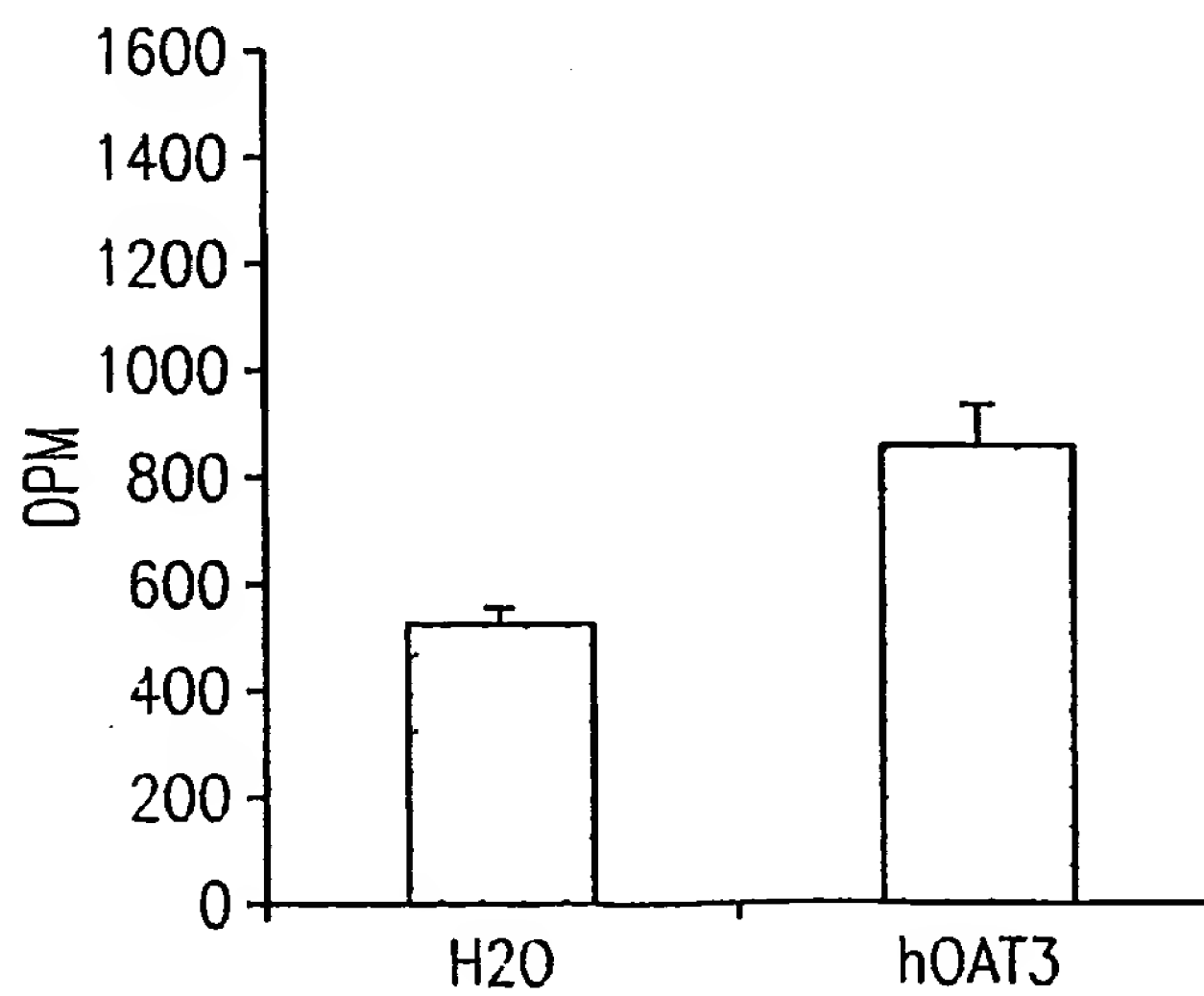
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*FIG. 9*

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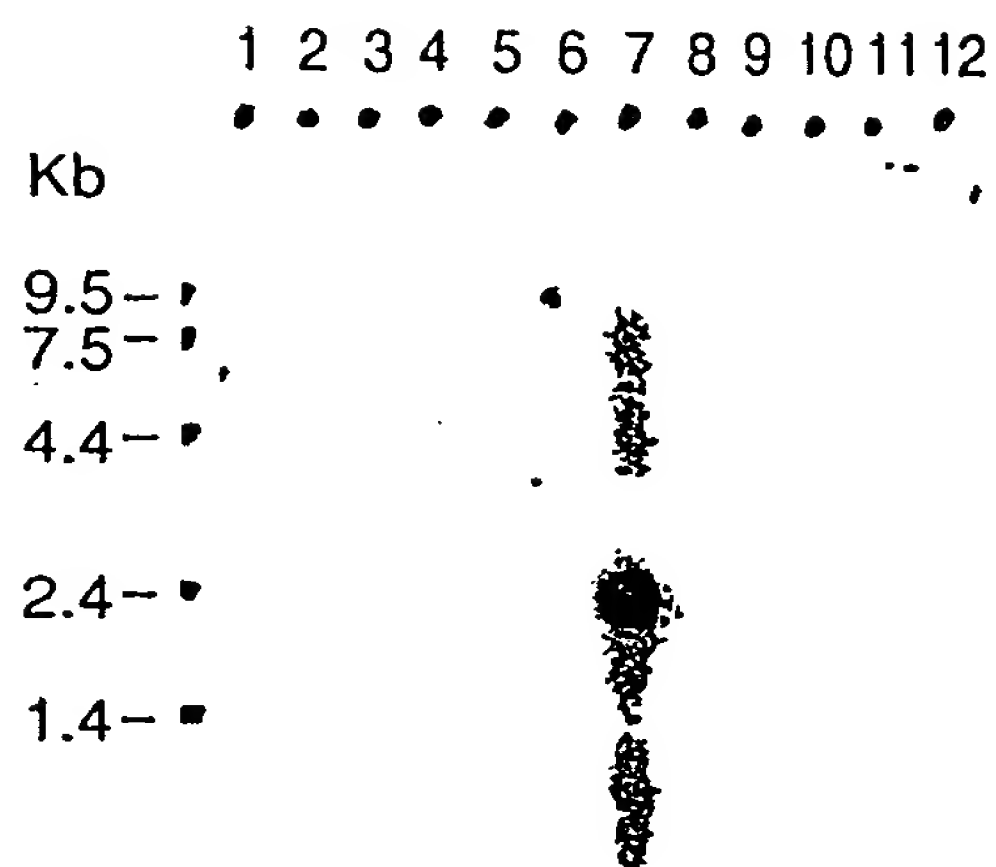
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## PAH TRANSPORT BY EcR293-OAT2A

**FIG. 11****FIG. 12**

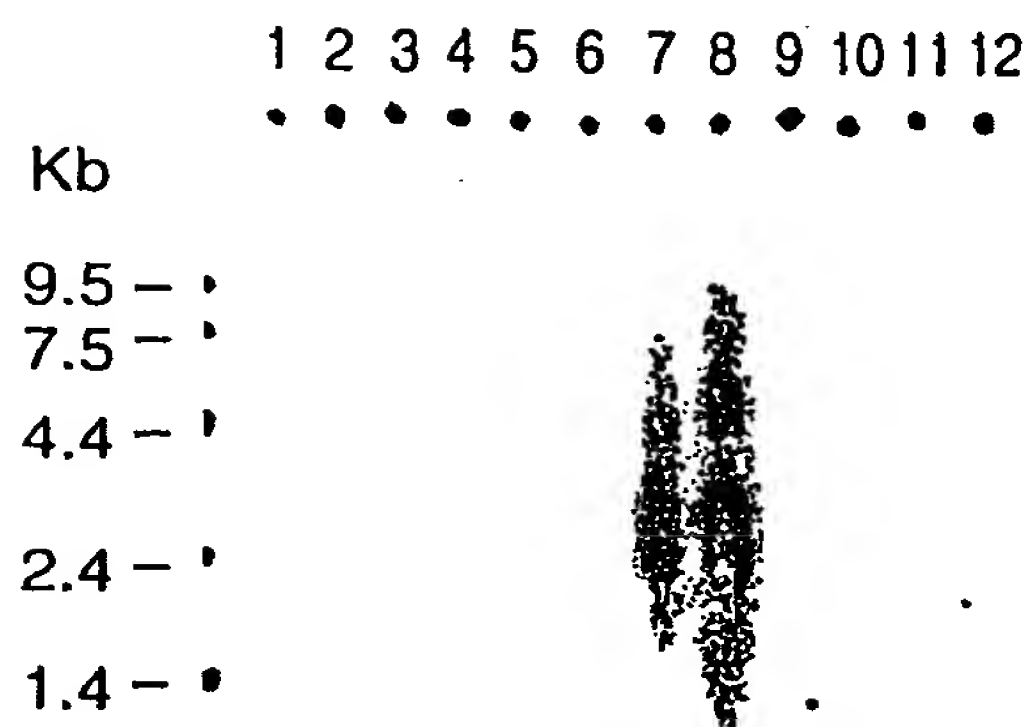
SUBSTITUTE SHEET (RULE 26)

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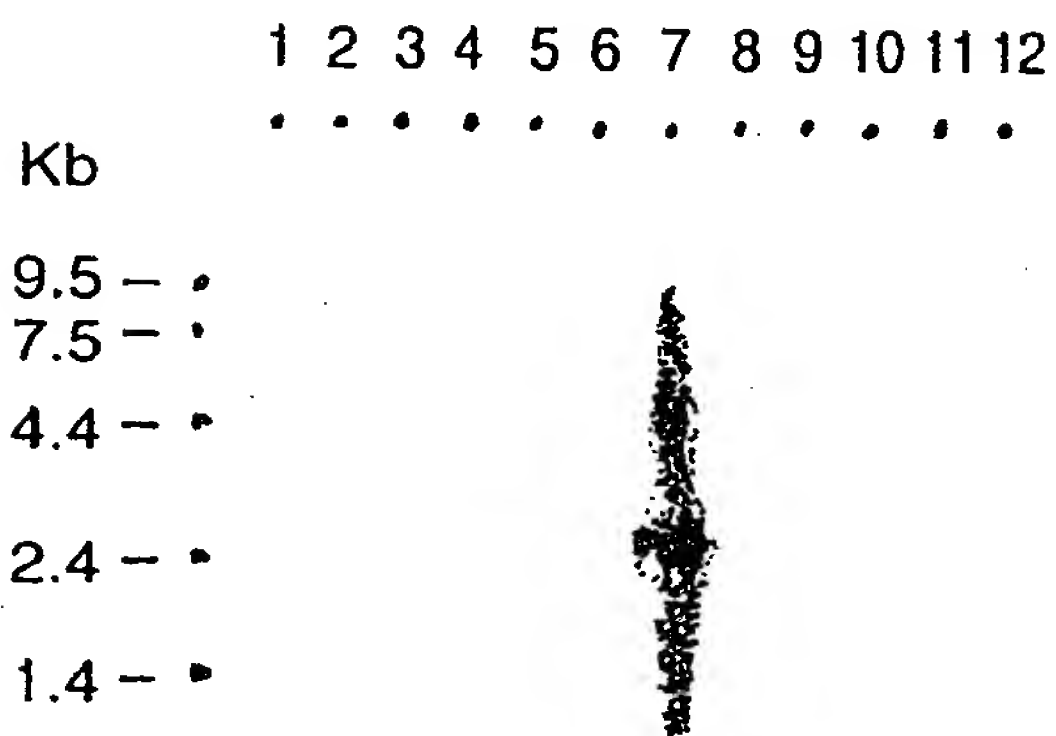
hOAT1

**FIG. 13A**



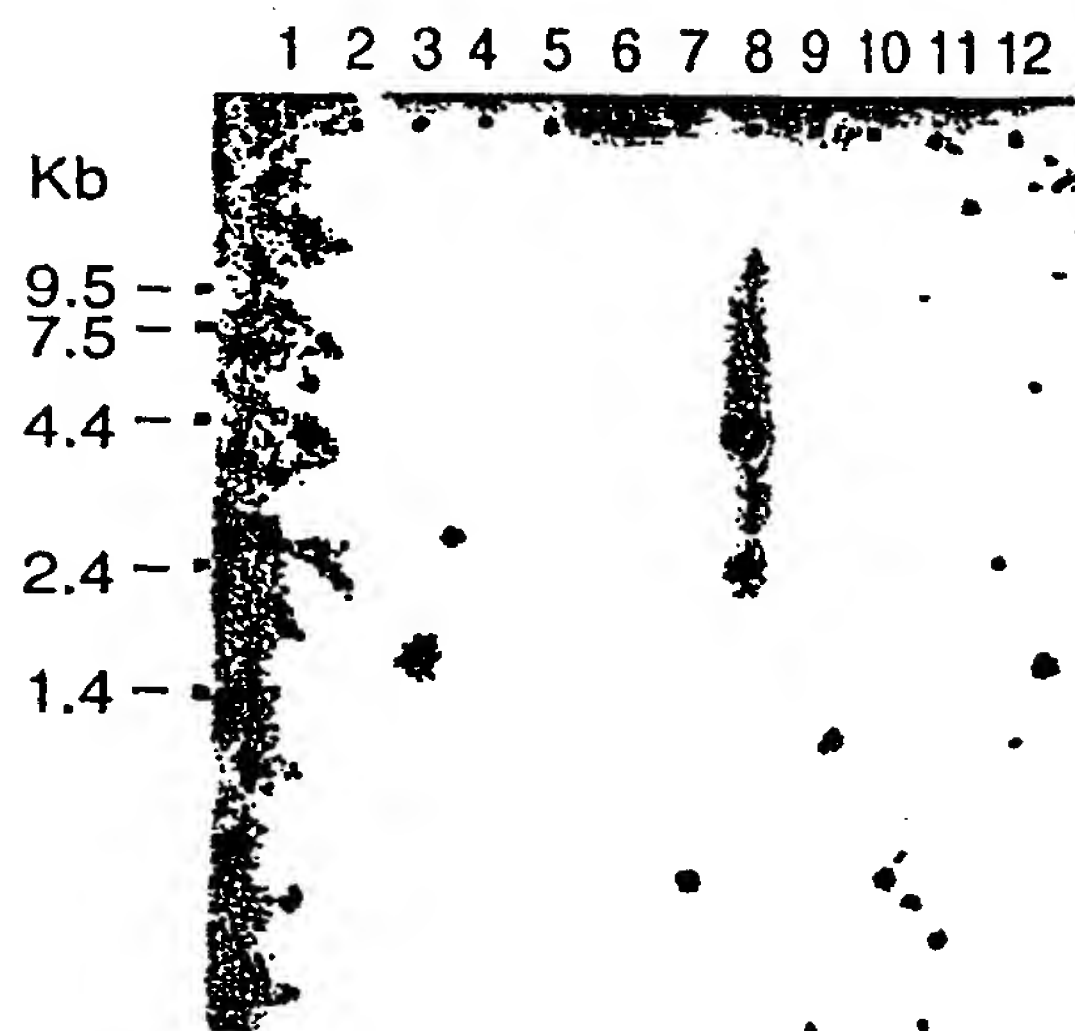
hOAT2

**FIG. 13B**



hOAT3

**FIG. 13C**

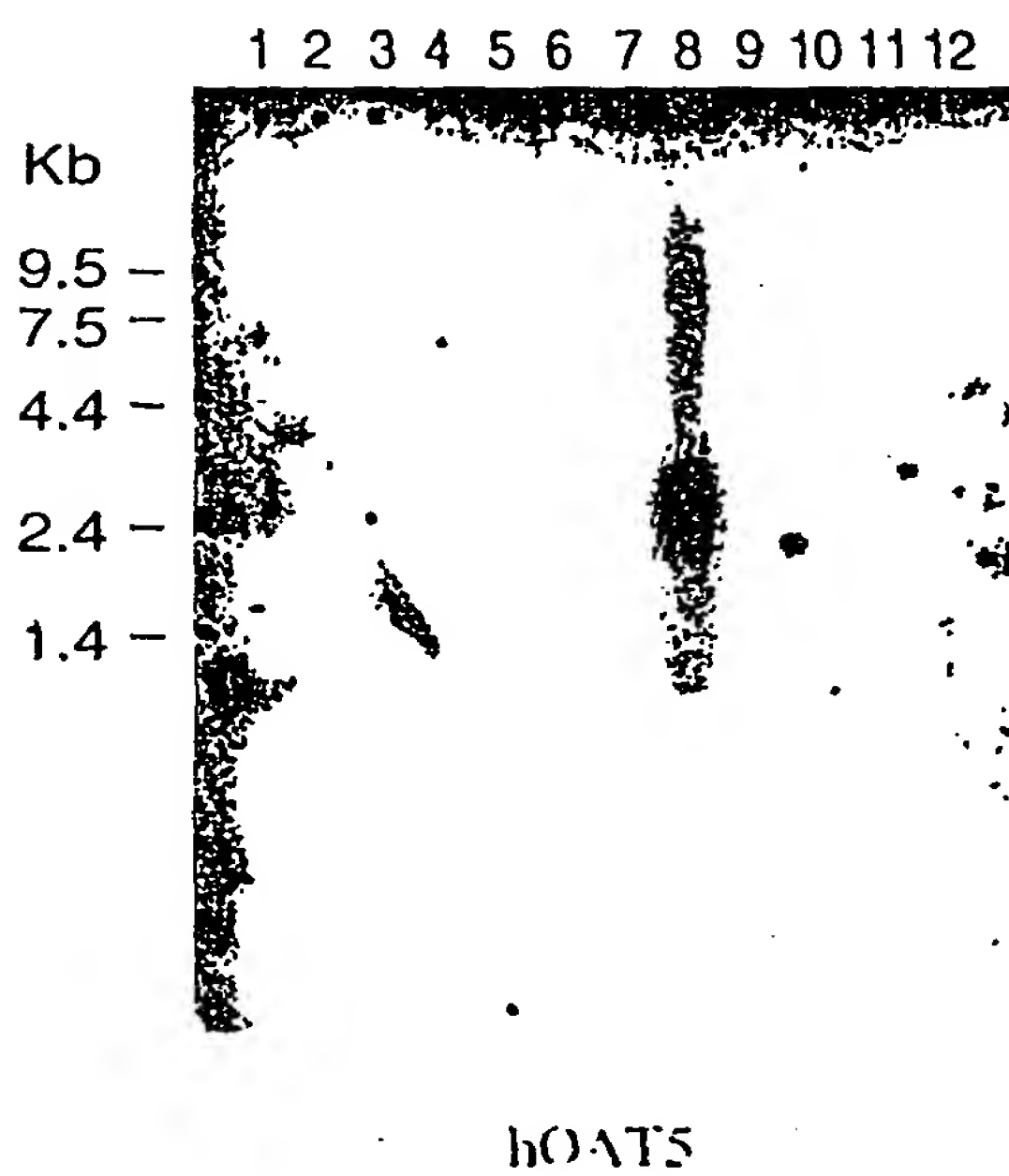


hOAT4

**FIG. 13D**



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***FIG. 13E***

## INTERNATIONAL SEARCH REPORT

International Application No

PL/US 00/18980

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 C12Q1/68 C07K16/28 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.                      |
|----------|---|--|
| X        | DATABASE EMBL [Online]<br>Accession number AF097518,<br>7 June 1999 (1999-06-07)<br>KIM R.B. ET AL. : "homo sapiens<br>liver-specific transporter (NLT) mRNA,<br>complete cds."<br>XP002156221<br>the whole document<br>---   | 1-5,9,<br>10,<br>14-16,<br>20,24-54        |
| X        | KUSUHARA HIROYUKI ET AL: "Molecular<br>cloning and characterization of a new<br>multispecific organic anion transporter<br>from rat brain."<br>JOURNAL OF BIOLOGICAL CHEMISTRY,<br>vol. 274, no. 19, 7 May 1999 (1999-05-07),<br>pages 13675-13680, XP002156219<br>ISSN: 0021-9258<br>the whole document<br>--- | 1-6,<br>9-11,<br>14-17,<br>20,21,<br>24-54 |
| -/--     |   |  |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

5 January 2001

Date of mailing of the International search report

06.02.01

Name and mailing address of the ISA

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Fax: (+31-70) 340 3016

Authorized officer:

Gurdjian, D

## INTERNATIONAL SEARCH REPORT

International Application No

PL./US 00/18980

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.     |
|----------|--|---------------------------|
| X        | DATABASE EMBL [Online]<br>Accession number AI016020,<br>18 June 1998 (1998-06-18)<br>ROBERT STRAUSBERG: " similar to TR:Q63314<br>Q63314 LIVER-SPECIFIC TRANSPORT PROTEIN"<br>XP002156222<br>the whole document  | 1-5,9,<br>10,<br>14-16,20 |
| A        | ---<br>WO 98 53064 A (HOSOYAMADA MAKOTO ;TANABE<br>SEIYAKU CO (JP); ENDOU HITOSHI (JP); SEK)<br>26 November 1998 (1998-11-26)<br>abstract<br>page 22-32  | 1-54                      |
| A        | ---<br>SIMONSON GREGG D ET AL: "Molecular<br>cloning and characterization of a novel<br>liver-specific transport protein."<br>JOURNAL OF CELL SCIENCE,<br>vol. 107, no. 4, 1994, pages 1065-1072,<br>XP000971769<br>ISSN: 0021-9533<br>the whole document<br>----- | 1-54                      |

# INTERNATIONAL SEARCH REPORT

national application No.  
PCT/US 00/18980

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3 ,14, 24-54 partly and 4,5,9,10,15,16,20

human organic anion transporter OAT2a and OAT2b with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

2. Claims: 1-3 ,14, 24-54 partly and 6,11,17,21 partly

human organic anion transporter OAT3 with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

3. Claims: 1-3 ,14, 24-54 partly and 7,12,18,22 partly

human organic anion transporter OAT4 with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

4. Claims: 1-3 ,14, 24-54 partly and 8,13,19,23 partly

human organic anion transporter OAT5 with corresponding amino acid and nucleotide sequences , nucleic acid probes , recombinant cells , antibodies , methods of identifying modulators and substrates amphibian oocytes and identification of toxic compounds ,

### Information on patent family members

PC./US 00/18980

Form PCT/ISA/210 (patent family annex) (July 1992)